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Contact: Chemistry-Biology Department, Technical University of Cluj Napoca, North Universitary Center of Baia Mare, Victoriei Str. 76, Baia Mare, Tel. 0040 262-276059; Fax: 0040262-275368;
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CONTENT

CARBON FOOT PRINT OF FOODS

M. Shakila Banu[1], P. Sasikala[1]

[1,2] *Department of Food Processing and Preservation Technology , Faculty of Engineering , Avinashilingam University For Women, Coimbatore.*

ABSTRACT

A 'carbon footprint' is a measure of the greenhouse gas emissions associated with an activity, group of activities or a product. The increasing interest in 'carbon foot printing' comes as a result of growing public awareness of global warming. The environmental impact of food consumption is of major concern, and efforts can be made to include greater environmental responsibility throughout the whole food chain, from cultivation, processing, transport and through to final cooking technique. A carbon footprint analysis can evaluate any of the life stages in the food production similar to a Life Cycle Assessment. The global community now recognises the need to reduce greenhouse gas emissions to mitigate climate change. Calculating a carbon footprint can be a valuable first step towards making quantifiable emissions reductions. A wider system boundary incorporating the entire life cycle from its production, distribution, consumption and disposal is a more holistic way to determine the carbon intensity of food. This paper presents an overview on carbon foot print of foods highlighting the carbon hotspots in the life cycle of food.

Keywords: *'carbon footprint', greenhouse gas emissions, foods, Life Cycle Assessment, carbon hot spots*

1. Introduction

'Carbon footprint' has become a widely used term and concept in the public debate on responsibility and abatement action against the threat of global climate change. It had a tremendous increase in public appearance over the last few months and years and is now a buzzword widely used across the media, the government and in the business world [1].

Figure 1. Carbon foot print

Concerns about greenhouse gas (GHG) emissions and their effect on global warming have inspired the quantification of the carbon footprint, i.e. the contribution to GHG emissions in carbon equivalents (CE) of many human activities [2]. The food processing sector is one which contributes significantly to the global carbon emissions, through agriculture, processing, packaging and transporting food.

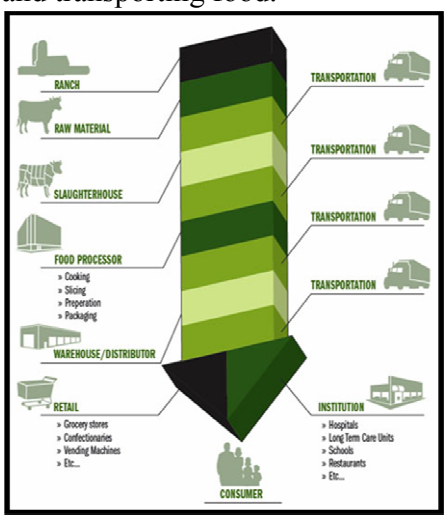


Figure 1. Food Chain

Food chains around the world are responsible for a large share of total emissions of greenhouse gases (GHG's) [3]. Food chain produces greenhouse gas (GHG) emissions at all stages in its life cycle, from the farming process and its inputs, through to manufacture, distribution, refrigeration, retailing, food preparation in the home and waste disposal. The carbon footprint provides a benchmark for measuring future reductions in greenhouse gases [3].

2. Life cycle assessment

A carbon footprint of food products is a powerful way to identify greenhouse gas emissions of from "cradle to grave"[4].

Figure 3. Life Cycle Assessment

A life cycle assessment of a product will help to [4]:

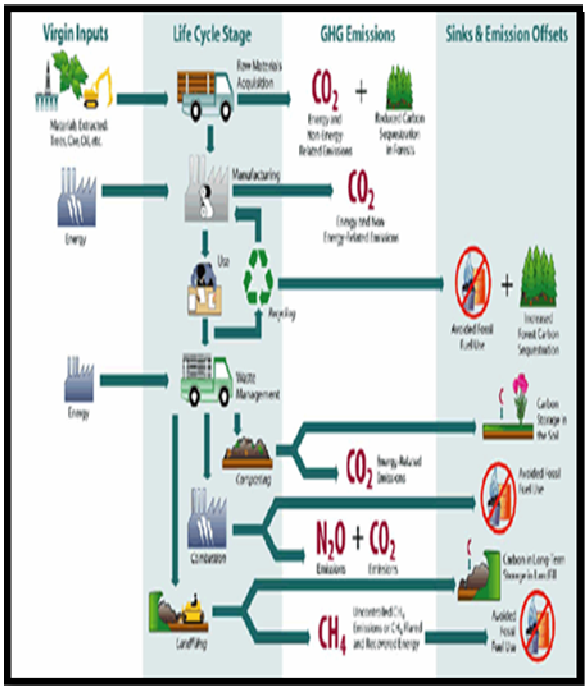


Figure 4. Carbon foot print in the Life Cycle Assessment [4]

Life-cycle thinking will help to understand how the products are contributing to climate change and where in the process the action should be made to reduce the climate impact [4].

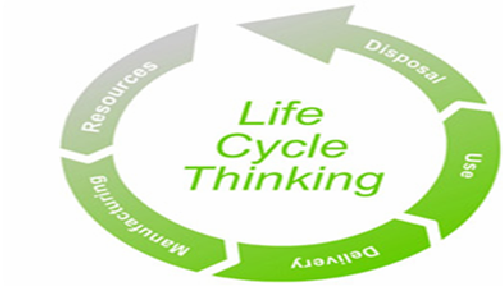


Figure 5. Life Cycle thinking

3. Food print as fraction of total foot print

Food-related emissions comprise 21% of total emissions, or 6.1 tons out of 28.6 tons per person per year. 15% of personal transportation relates to food, as does 20% of housing energy use, while 23% of the emissions from all other activity are food-related [5].

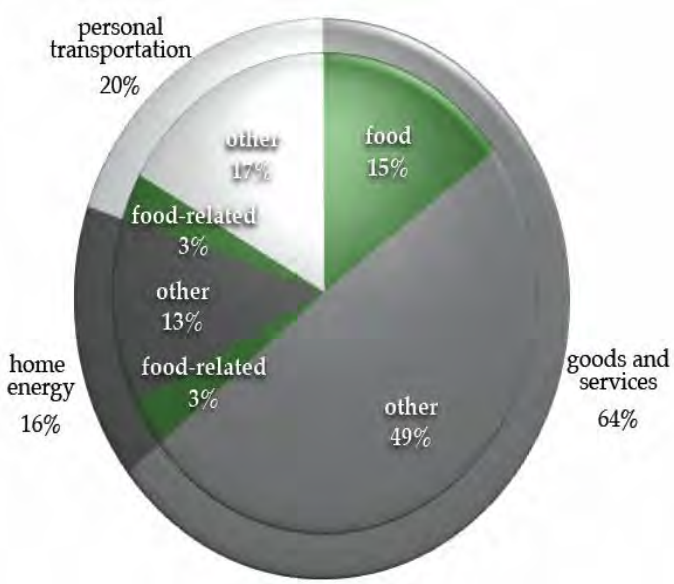


Figure 6. Food print as fraction of total footprint [5]

3. Carbon foot print in the food system

The carbon foot print of food refers to the amount of greenhouse gases produced from growing, processing, packaging and transporting food.

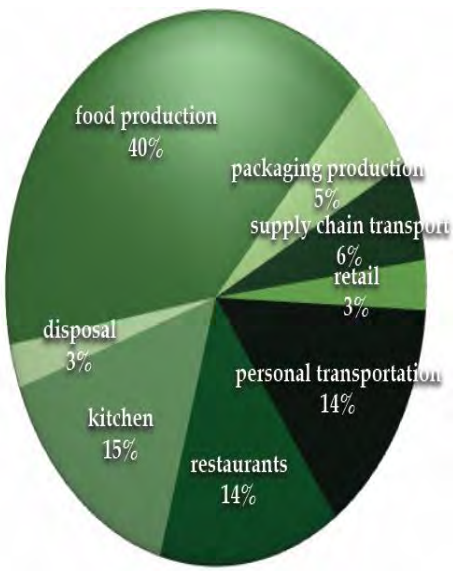


Figure 6. Breakdown by lifecycle phase [5]

The food system contributes significantly to global GHG emissions [2]. All stages in the supply chain contribute, but on average the agricultural stage is the single biggest GHG emitter, while meat and dairy products are the most GHG-intensive food types [2].

Reducing total global consumption of animal products (meat and dairy foods) can lead to reduced CO_2 and methane emissions produced by animals. Using more locally produced seasonal products reduces "food miles" and uses less fossil fuel in delivery [6].

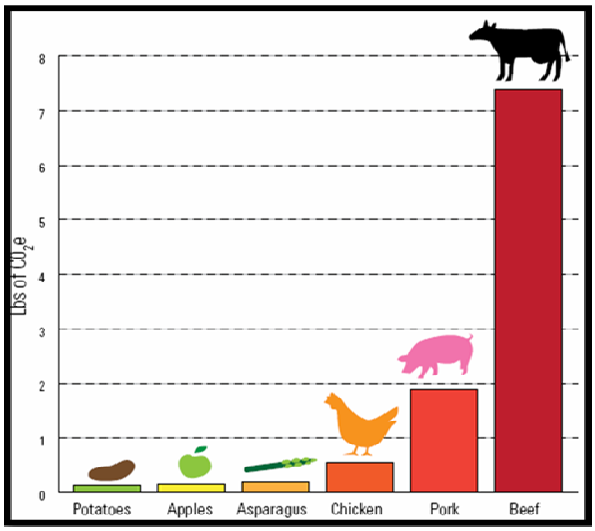


Figure 7. Global warming pollution released by foods [7]

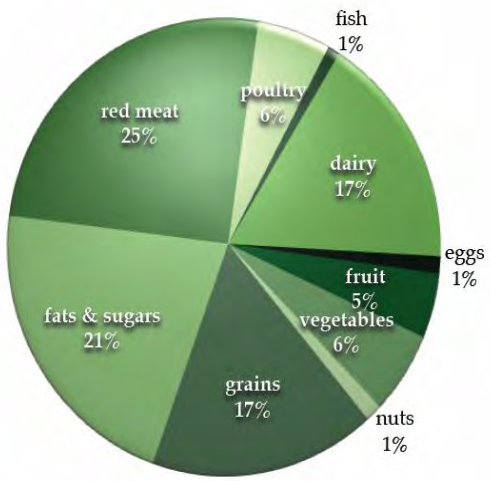


Figure 8. Breakdown by food groups [5]

From Figure 8, it can be observed that a quarter of the average food print derives from red meat, with another quarter coming from other animal products. Plant foods make up the remaining half, of which the majority results from grains, fats, and sugars.

4. Green house gases in food emissions

Three main gases comprise the vast majority of food-related emissions: carbon dioxide, methane, and nitrous oxide.

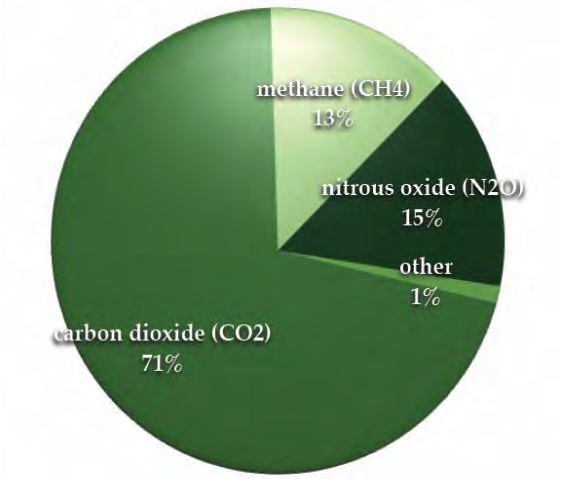
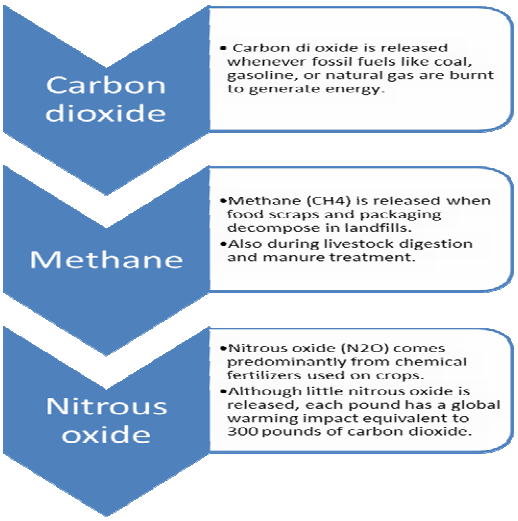


Figure 9. Breakdown by green house gases [5]

The majority of food's global warming impact comes from carbon dioxide, released during fossil fuel combustion. The remainder comes mostly in the form of methane and nitrous oxide, coming largely from livestock and chemical fertilizers, respectively.

Other gases, including SF_6 from electricity production and HFCs from refrigeration systems, comprise the remaining 1% [5].



5. Carbon footprint in each stage of food chain

5.1 Agriculture

Agricultural emissions account for 13 per cent of total GHG emissions, or between 5 and 6 gigatons (Gts) of CO_2 equivalents and they are predicted to rise by almost 40 per cent by 2030 (Smith et al., 2007). This is largely due to increased demand from a growing population and to a greater demand for ruminant meats.

Nitrous oxide is emitted mainly from fertilizer and manure applications to soils, while methane is emitted mainly in livestock production (fermentation in digestion), rice production and manure handling. Transport, processing, retailing and household consumption of food adds further emissions associated with agriculture [8].

5.2 Processing of foods

The food life cycle can be highly complex, with multiple processes.

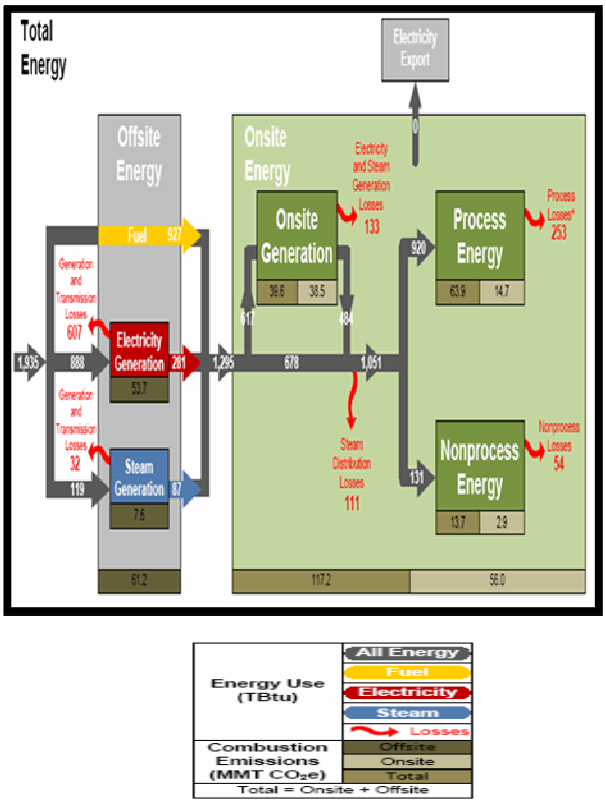


Figure 8. Manufacturing energy and carbon footprint (Beverage sector)
Source: 2006 MECS

For a food product, there may be a production process for nitrogen fertiliser, another for growing a crop or producing an animal for meat, another for making a ready meal, another for retail, and another for meal

preparation. To arrive at a carbon footprint (or to carry out a more comprehensive LCA) it is practical to perform separate assessments for each process, with some processes providing raw materials for processes further on in the life cycle [9].

5.3 Food transportation

"Food miles" is a term that is used to draw attention to the ecological cost of how far food travels from producers to consumers. Transportation is an important factor in evaluating the environmental impact of food.

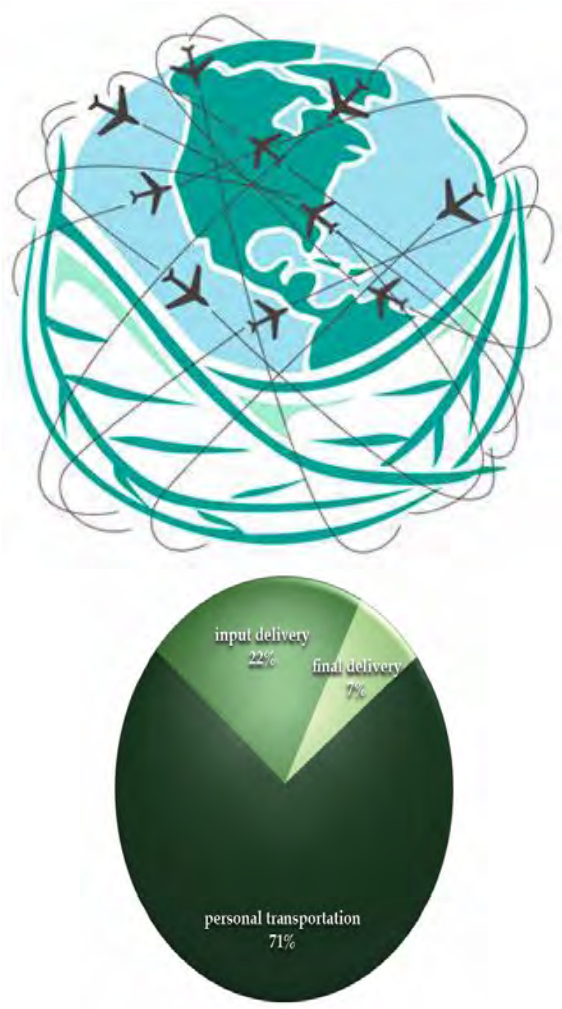


Figure 9. Sources of food transportation emissions [5]

Food miles refer only to the transport aspect of food products which is a narrowly defined system and only a small contribution to the overall carbon footprint of the product. Food miles have become one method for evaluating the sustainability of the global food system in terms of energy use. This concept has received an increasing amount of attention over the last decade as climate change patterns have become ever more apparent [10].

Figure 10. Food miles

5.4 Dining in and eating out

Producing and transporting ingredients accounts for the majority of food-related emissions, but the energy to transform those ingredients into a steaming meal is an important contributor as well. Taking into account storage, cooking, and cleanup, kitchen energy use amounts to 15% of the total food-related emissions. Home kitchens account for 1,850 pounds of CO_2 per person annually, or 15% of the average food footprint, while food service (including restaurants and cafeterias) accounts for another 14%, or 1,740 pounds. In all, the energy to cook and serve a meal accounts for nearly a third of its entire life cycle emissions.

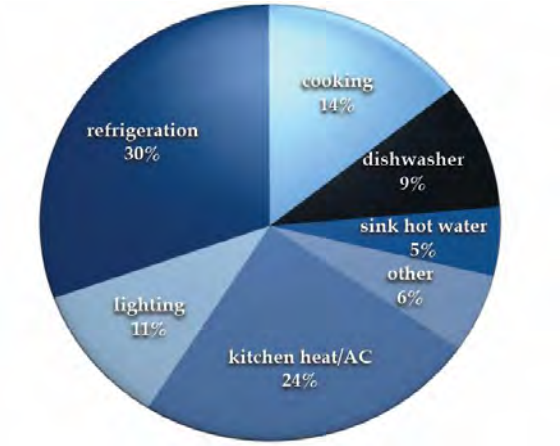


Figure 11. Home kitchen energy emissions

6. Benefits of carbon footprint assessments

Carbon footprint assessments can be used in many ways, for example, to:[9]

- Inform strategies for decreasing a carbon footprint
- Satisfy customer and/or investor demands
- Compare alternative production methods
- Define industry benchmarks
- Identify hotspots
- Encourage best practice
- Improve business performance (e.g. through energy saving or competitive advantage)
- Label a product with an emissions value.

7. Outlook

Carbon foot printing can provide valuable insights into the life cycles of food products and their environmental significance. The food system contributes significantly to global GHG emissions. Technological improvements, while essential, will not be sufficient in reducing GHG emissions. The combination of population growth and rising per capita anticipated consumption of meat and dairy products will undermine the cuts that technological and managerial innovation can achieve.

Finally, while GHG mitigation is important, it is not the only priority. Measures to reduce food chain GHG emissions need to be assessed within the context of other social and environmental concerns. These include human nutrition, biodiversity, water use and animal welfare. For any given mitigation measure, decision makers will need to consider the extent to which it moves us away from, or towards, achieving a more resilient, healthful, and morally attentive system of food production and consumption.

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TESTING THE PRESERVING ACTIVITY OF NANOSTRUCTURED Ag-TiO_2 DURING THE DEPOSITION OF SUMMER SAUSAGE AND BONELESS CHICKEN BREAST

Adela But[1*], Andrea Bertoti[1]

[1] *Technical University of Cluj Napoca, North University Center of Baia Mare, Department of Chemistry and Biology, Victor Babes 62A, 430083, Romania, phone: 004-0744-790308, *peteranca@yahoo.com*

ABSTRACT

The preserving activity of different types of packages containing nanostructured Ag-TiO_2 was established during the deposition of summer sausage and boneless chicken breast, at room temperature, by monitoring the organoleptic (colour, aspect, consistence, flavour) and physico-chemical (dry mass, chloride and protein contents, pH) characteristics of the food samples. The morpho-structural analyses (X-ray diffraction, FTIR spectroscopy and TEM microscopy) of Ag-TiO_2 composite demonstrated the crystalline structure, containing anatase in the mean composition, together with small quantities of rutile. The TEM micrograph showed the heterogeneous structure of the composite. The results demonstrated the higher efficiency of the package with Ag-TiO_2 in preserving the investigated food samples as compared to the conventional packages, in terms of preserving the water, chloride and protein contents and of pH. The results showed that in the chicken breast and the summer sausage kept in F-Ag-TiO_2-F package, the decrease of the water, chloride and protein contents and of pH were the most reduced. So, this type of package is an alternative to the conventional packages to preserve the quality of the meat products.

Keywords*: Ag-TiO_2, active packages, summer sausage, chicken meat*

1. Introduction

The study of the nanomaterials as active packages in food industry represents a recent research direction [1], with the aim of improving the mechanical and isolator properties, biodegradability as compared with the conventional polymers used as package. The nano-packages are studied also for their antimicrobial activity.

Titanium dioxide is widely used in many processes such as: depollution, syntheses [2-11] and more recently, in food industry incorporated in active packages and in nano-sensing systems for monitoring the food quality and safety [12]. The waste application of TiO_2 is based on its photocatalytic activity generated by excitation of the TiO_2 particles, when charge carriers are formed. The holes remain in the valence band (VB) and initiate oxidation reactions and electrons jump in the conduction band (CB) and initiate reduction reactions [13]. All these reactions achieve the preserving and antimicrobial activity of TiO_2. By modifying the TiO_2`s surface with Ag nanoparticles, new materials excitable by the solar light are obtained.

Li et al. have demonstrated that the Jujuba fruits packaged in bags containing TiO_2 have a long shelf life, by diminishing the degradation and browning rates as compared with the fruit deposited in conventional package [2]. Karatapanis et al. have demonstrated that the milk deposited in package with nanostructured TiO_2, for 7 days, contains a high quantity of vitamin A than the milk deposited in the conventional package. Moreover, the Chinese cabbage kept in package with TiO_2 during 10 days, showed a lower mass lost than the sample kept in conventional package [14-17].

The aim of this study is to establish if the packages with Ag-TiO_2 are more efficient in preserving the summer sausage and chicken breast than the conventional ones.

2. Materials and methods

2.1. Preparation of Ag-TiO_2

First the TiO_2 gels were prepared via sol-gel method, by acidic hydrolysis of titanium tetra-izopropoxide (TIP). The molar ratios of the reactants was: [IPT] : [H_2O] : [C_2H_5OH] : [HNO_3] = 1 : 3,325 : 19 : 0.08.

The transparent gel was allowed to age 4 weeks to finallize to polimerization and condensation reactions and then is immersed in solution containing Ag ions (0.005M), for 24 h. Then the gels were washed with ultrapure water, to eliminate the Ag ions in exces. Then, the gels were dried for 24 h, at 80^0C and calcined at 500^0C, 2h, the heat rate being 4^0C/min. The obtained composite was used to the packages preparation.

2.2. Packages preparation

A suspension of crystallized Ag-TiO_2 (0,05 g) in ethanol (2 ml) was prepared and coated on 400 cm^2 polyethylene film (Supermarket line Kaufland Romania), using a knife, and dried in air for 10 minutes. Packages, in which, a second polyethylene film was deposited onto Ag-TiO_2 were also prepared.

In the as obtained packages, 50 g food sample (summer sausage or boneless chicken breast acquisitioned from the Supermarket line Kaufland Romania) was measured and packed. In the packages with the structure polyethylene- Ag-TiO_2 the food sample is not in contact with Ag-TiO_2 and in the packages with the structure polyethylene-Ag-TiO_2-polyethylene the food sample is not in contact with Ag-TiO_2.

The bags were maintained, at $22,5^0$C, for allowing the visible light to excite the TiO_2 particles.

At different periods of time, humidity, chlorine and protein content and pH, analyses were made. Additionally, organoleptic analyses were performed for the food samples.

2.3. Orgaloleptic analyses consisted in establishing the variations of color, flavour, aspect and consistence of the food samples deposited in studied packages, at different time ranges.

2.4. Humidity

A sample of 5 g (G) was mixed with calcined cuartz sand and the mixture is weight (G_1). The mixture was then treated with 5 ml of ethanol (S.C. Chemical Company S.A. Iaşi, România) and introduced in the Binder oven at 40...60°C, for 2 h and then at 105...110°C, until the weigh is constant [18] (STAS 961-56). After cooling the mixture is weigh again (G_2). The percentage of water is calculted with the formula:

$$\%water = \frac{G_1 - G_2}{G} \times 100 \qquad (1)$$

when : G_1 - mass of food sample and sand before drying (g),

G_2 - mass of food sample and sand after drying (g)

G - mass of food sample (g)

2.5. Chlorine content

A sample of 5 g was mixed with 15 ml acetone and then 50 ml of ultrapure and a small quantity of sodium carbonate were added. The mixture was filtered and the entirely quantity of extract was added to 0.5 ml of potassium chromate 10% (S.C. Chemical Company S.A. Iaşi, România) were added. The mixture was titrated with 2.906% solution of silver nitrate (S.C. Chemical Company S.A. Iaşi, România) until the color changed in red-brown [18] (STAS 961-56) .

The chlorine content was calculated using the formula:

$$\%NaCl = \frac{V}{m} \qquad (2)$$

where: V – volume of $AgNO_3$ solution used to titration (ml).

m – sample mass (g)

2.6. pH

A sample of 5 g was mixed togheter with 30 mL ultrapure water and then filtered. In the obtained filtrate the pH was measured using the Inolab pH 730 pH-meter.

2.7. Protein content

In an Erlenmeyer vessel, 5 g of sample are mixed with 2 ml potassium oxalate solution 2%. Then, 1 ml phenolphthalein 2% was added. The mixture was titrated with NaOH solution 0.143 N until the pink colour appeared, for the free acidity neutralization.

Then, 10 mL formaldehyde solution was added and the mixture was stirred. The pink colour disappeared. The mixture is let to rest for 30 seconds and then is titrated again with NaOH until the pink color appeared [18] (STAS 6355-61). The protein content is calculted as follows:

$$protein\ (\%) = \frac{V}{2} \qquad (3)$$

where: V- volume of NaOH 0,143 N (ml) used to the second titration

All the physico-chemical analyses were repeated three times and the standard deviation was calculated using the Microsoft excel software.

2.8. Structural and morphological characterisation of Ag-TiO_2

The FTIR analysis was performed on a Perkin Elmer FTIR 2000 instrument (2010). Sample wafer consisted of 100 mg spectral quality KBr and ca. 1 mg composite.

The crystalline structure and the crystallite size were determined from the X-ray diffraction (XRD) patterns recorded with a Shimadzu XRD-6000 diffractometer, using Cu K_α radiation (λ=1.5418 Å), with Ni-filter.

A Jeol JEM1010 (Japan) Transmission Electronic Microscope operating at an accelerating voltage of 80 kV, coupled with an images acquisition system MegaView III CCD Camera (1.2Mp) Soft Imaging Solutions (Olympus) was employed to obtain TEM images.

The concentration of Ag in the composites was established using the Atomic Absorption Spectrometer AAS 800 Perkin Elmer was 0.21 ± 0.01%.

3. Results and disscussion

3.1. Organoleptic analysis

The reference chicken breast and summer sausage samples (0 day) are fresh, the shells are dried, without microorganisms or slime, adherent to the compostion. The colour of the samples was pink, uniformous, without stains, the consistence was dense and juicy, the flavour was nice, characteristic to that of the fresh chicken meat and summer sausage.

Alter 8 days of storage, the chicken meat deposited in polyethylene (F) became soft, in some regions, slime was observed. The colour became light pink and the flavour was pungent. The chicken breast deposited in package with Ag-TiO_2 was also soft but the flavour was not so altered as that of the meat deposited in polyethylene only (Figure 1 a).

After 8 days of storage, the summer sausage deposited in the polyethylene package became brown, in the entirely sample (surface and bulk) and the flavour is deeply altered. In comparison, the colour of the sample kept in package with Ag-TiO_2 was almost the same as to that of the reference sample, and the flavour is not so altered as that of the sausage kept in polyethylene (Figure 1 b).

3.2. Physico-chemical parameters

Table 1 presents the physico-chemical parameters of the food samples deposited in packages containing Ag-TiO_2.

The water content of the chicken breast kept in polyethylene decreased after 4 days and then increased.

In the F-Ag-TiO_2-F package, the water content of the chicken breast increased from 62.56 % up to 70,76%, and then decreased. In package F-Ag-TiO_2-Ag, the humidity of the chicken breast has slighly decreased, but after 8 days of storage, increased up to 73,2%. The humidity of the chicken breast varied in the most lower manner in F-Ag-TiO_2 package.

The water content of the summer sausage has been changed during deposition in the packages with Ag-TiO_2 in 8 days of storage. The water content of the summer sausage kept in polyethylene and F-Ag-TiO_2 decreases drastically as compared with that of the sample kept in F - Ag-TiO_2 – F, due to the „sandwich" structure of that package, which assure a barrier that inhibit the water evaporation.

The chloride content (Table 1) of the chicken breast deposited in all the investigated packages decreases from 0.6 % (0 day) to 0.23 % in polyethylene, 0.14 % in F-Ag-TiO_2 and 0.28 in F-Ag-TiO_2-F, after 8 days of storage. This decrease is due to the photolysis (in polyethylene) and photocatalysis (in packages with TiO_2). The photolysis is defined as the degradation process catalysed by light only. During these processes, the chloride ions are oxidized by the holes, generating chloride radicals and, subsequenlty, the chloride radicals are coupled, thus forming molecular chlor, which leaves the system (reactions 1 and 2).

$$h^+ + Cl^- = Cl^* \quad (1)$$

$$Cl^* + Cl^* = Cl_2 \quad (2)$$

The decrease of the chloride content in the summer sausage kept in polyethylene is the most reduced after 4 days of storage (from 4,40 % to 2,20%), as compared with the other packages (from 4,40% to 2,10 in the F - Ag-TiO_2 – F and from 4,40% to 2,07% in F - Ag-TiO_2). After 8 days of storage, the chloride content drastically decreases from 2,20% to 1,21 % in polyethylene, to 1.8 % in F-Ag-TiO_2-F and to 1.26 % in F-Ag-TiO_2). The most reduced decrease was observed for the summer sausage kept in F - Ag-TiO_2 – F, after 8 days of storage [1].

The pH of the chicken breast increased in all packages during 4 days of storage. After 8 days, the pH of the sample in polyethylene increased due to the generation of the acidic organic compounds in the fermenation processes, but the pH of the sample in F-Ag-TiO_2-F and F-Ag-TiO_2 decreased due to the photocatalytic degradation of the acidic compounds generated during the meat fermentation [1, 13, 16].

The pH of the summer sausage kept in all packages decreases due to the fact that the degradation of the active principles in sausage occurs during storage. The most reduced acidification of the sample was observed during storage in F - Ag-TiO_2 – F, due to the properties of the Ag-TiO_2 composite, such as: photocatalytic activity, that induces the degradation of the acidic compounds and low permeability for oxigen, which reduces the rate of oxidation in the food product [1].

The protein content of the chicken breast kept in all the packages decreased during storage, due to the photolysis and photocatalytis processes occuring in polyethylene package and nano-packages, respectively. After 4 days of storage, the protein content decreased from 6.73 % (reference) to 6.5 % in F, to 6 % in F-Ag-TiO_2-F and to 4.9 % in F-Ag-TiO_2. After 8 days of storage, the protein content decreased to 3.25 % in F, to 4 % in F-Ag-TiO_2-F and to 4.4 % in F-Ag-TiO_2. Thus the package in which the chicken breast had the highest protein content was F-Ag-TiO_2.

In the case of summer sausage, the most efficient package, in which the sample had the highest protein content after 8 days of storage, was F - Ag-TiO_2 – F.

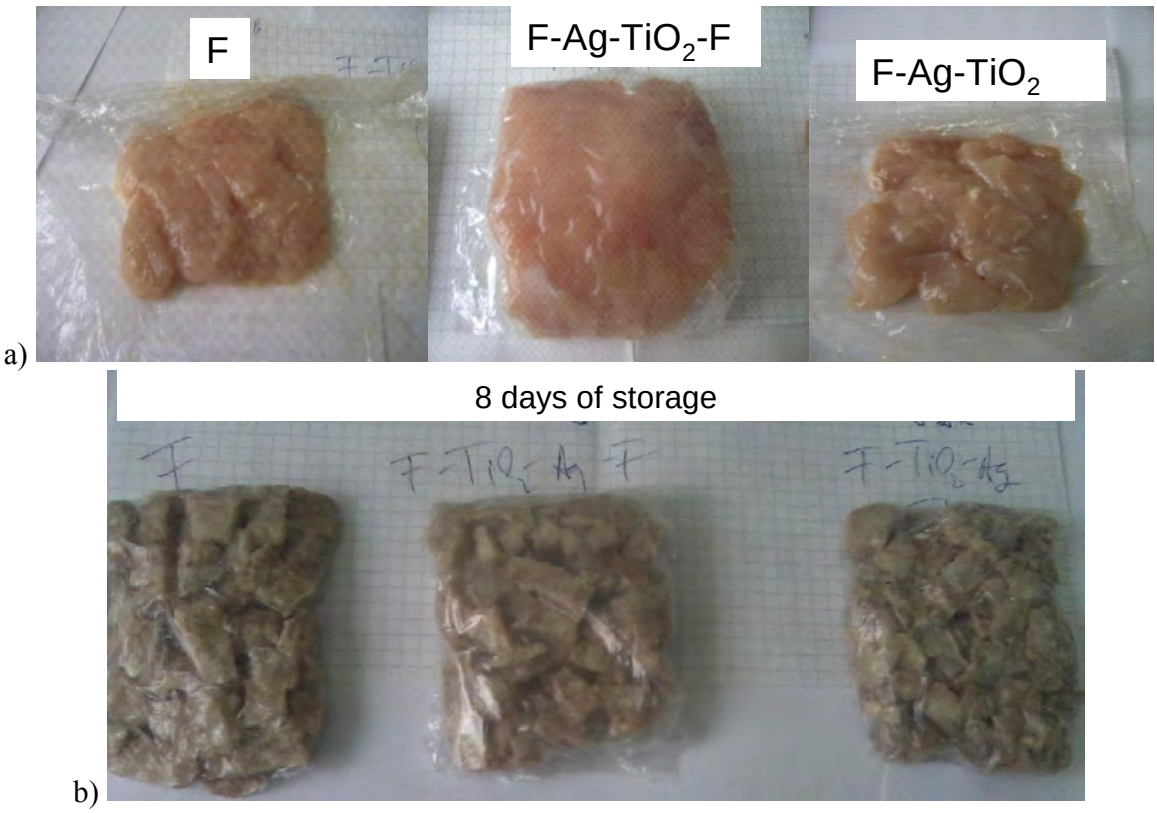

Figure 1. Images of the chicken breast a) and summer sausage b) samples kept in different packages during 8 days (F-polyethylene)

Table 1. *Physico-chemical parameters of the food samples kept in different packages (F-polyethylene, SD – standard deviation)*

Period	**0 day**				**4 days**				**8 days**			
Summer sausage												
Packa-ge ID	**NaCl [%] ±SD**	**pH ±SD**	**water [%] ±SD**	**Protein [%] ±SD**	**NaCl [%] ±SD**	**pH ±SD**	**water [%] ±SD**	**Protein [%] ±SD**	**NaCl [%] ±SD**	**pH ±SD**	**water [%] ±SD**	**Protein [%] ±SD**
F	4.40 ±0.10	7.87 ±0.01	64.96 ±0.25	9.61 ±0.05	2.20 ±0.10	7.13 ±0.01	54.80 ±0.25	5.55 ±0.05	1.21 ±0.02	6.59 ±0.07	45.82 ±0.61	4.41 ±0.18
F-Ag-TiO_2-F					2.10 ±0.03	7.36 ±0.02	58.80 ±0.30	6.83 ±0.15	1.80 ±0.07	6.67 ±0.05	51.7 ±0.95	6.50 ±0.15
F-Ag-TiO_2					2.07 ±0.03	7.17 ±0.01	52.70 ±0.04	6.26 ±0.25	1.26 ±0.05	6.29 ±0.35	49.80 ±0.69	5.04 ±0.04
Boneless chicken breast												
F	0,6 ±0,1	5,84 ±0,07	62,56 ±0,63	6,73 ± 0,56	0,40 ±0,04	6,3 ±0,02	62,58 ±0,62	6,50 ±0,4	0,23 ±0,03	6,68 ±0,36	67,86 ±0,06	3,2 ±0,02
F-Ag-TiO_2-F					0,43 ±0,02	6,75 ±0,10	70,76 ±0,12	6,00 ±0,05	0,28 ±0,02	6,63 ±0,04	62,8 ±0,06	4,4 ±0,45
F-Ag-TiO_2					0,51 ±0,26	6,53 ±0,04	56,43 ±0,65	4,9 ±0,25	0,14 ±0,01	6,54 ± 0,5	73,2 ±0,15	4,00 ±0,1

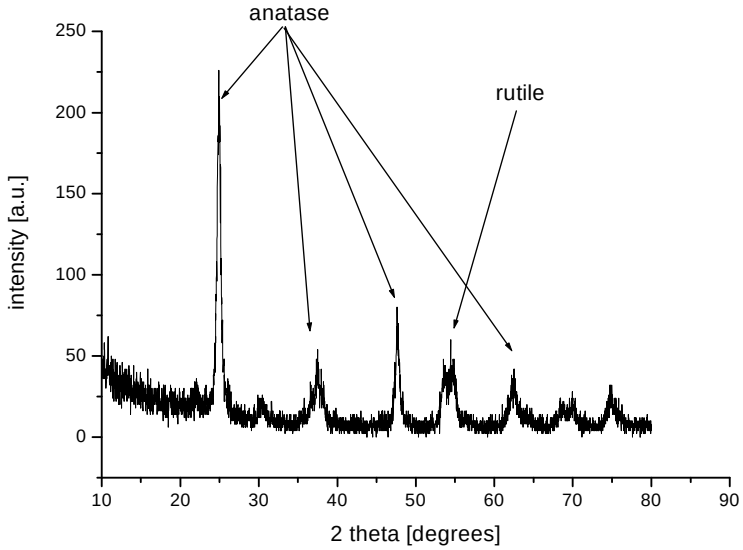

Figure 2. X-ray diffraction pattern of Ag-TiO_2

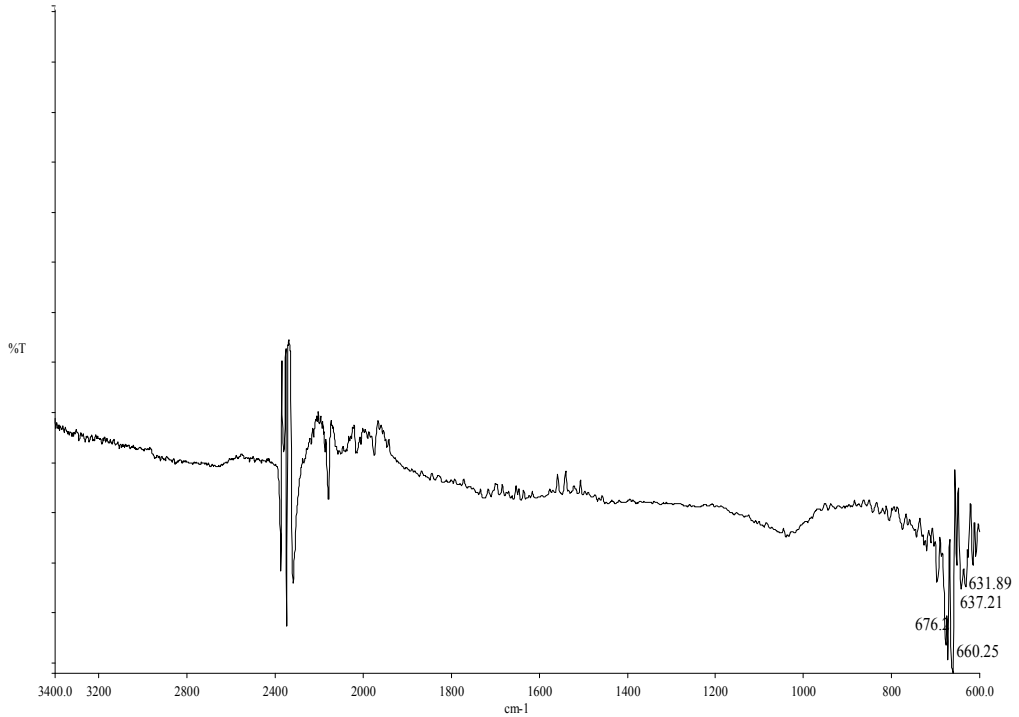

Figure 3. FTIR spectrum of Ag-TiO_2

3.3. Morpho-strucural characterisation of Ag-TiO_2 composite

The X-ray diffraction pattern of Ag-TiO_2 (Figure 2) demonstrates the crystalline structure of the composite, predominating the anatase phase. The peaks at $2\theta = 25$, 37.5, 47.5 and 62.5^0 are assigned to the anatase. A small peak at $2\theta = 53^0$ is assigned to the rutile phase [19]. No peaks assigned to the Ag particles was observed, due to the low content in the composite. The mean average of the anatase particles is 14 nm.

In Figure 3 is presented FTIR spectrum of Ag-TiO_2 in the range 600-3400 cm^{-1}. The peaks in the 2300–2400 cm^{-1} region are assigned to the acidic groups [8]. The small bands in the region 1550 - 1400 cm^{-1} more pronounced for TiO_2 than for the composites were attributed to stretching and vibrations of the Ti-O-Ti group.

The bands between 750 and 670 cm^{-1} are assigned to the Ti-O vibration in anatase nano-TiO_2 [1, 8].

TEM micrograph (Figure 4) indicates the heterogeneous structure of the Ag-TiO_2 composite. The TiO_2 particles are homogeneously dispersed, but the Ag particles, in some areas, are agglomerated in clusters and in other zones are isolated.

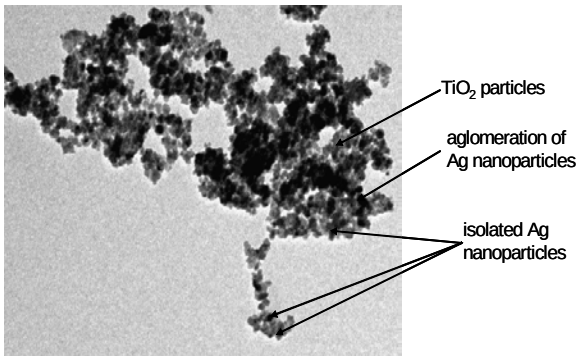

Figure 4. TEM micrograph of Ag-TiO_2

4. Conclusions

The results showed that in the chicken breast and the summer sausage kept in F-Ag-TiO_2-F package, the decrease of the water, chloride and protein contents and of pH were the most reduced. So, this type of package is an alternative to the conventional packages to preserve the quality of the meat products.

Our future intention is to investigate the preserving activity of the packages with Ag-TiO_2 during deposition of the meat products, in illuminated refrigerators.

5. Acknowledgement

We conducted most of the research work in the Scientific Research Center for Environment, Food and Health Safety, North University Center of Baia Mare, Technical University Cluj-Napoca.

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STUDY ON RED WINES CONTAMINATION WITH DIFFERENT TYPES OF MOULDS

Ioannis Vagelas[1*], Nikolaos Gougoulias[1], Liviu Giurgulescu[2], Arusti Raluca[2]

[1]*Technological Educational Institute of Larissa, Department of Plant Production, 41110 Larissa, Greece, vagelas@teilar.gr*

[2] *Technical University of Cluj Napoca, North University Center of Baia Mare, Department of Chemistry and Biology, Victor Babes 62A, 430083, Romania*

ABSTRACT

Romania is one of eastern Europe's major wine-producing nations and one of the ten largest wine exporters in the world. Wine production in the area of present-day Romania has a an ancient tradition going back over 6,000 years. The climate is mainly continental. Of the total vineyard area, 79 percent is to be found in Moldavia, Muntenia (Greater Walachia) and Oltenia (Little Walachia) regions. International red wine varieties such as Cabernet Sauvignon, Merlot and Pinot Noir are grown for example în Dealu Mare („the big hill'') within the Muntenia and Oltenia regions. The remaining wine-producting regions are centered around Transylvania. Romanian producers can be divided into two groups: the private producers, who have no funds for the necessary investment and simply sell their wine în canisters, and the state wineries. All this wineries have managed to raise the quality of their wines within a short space of time, albeit using international grape varieties. An increasing number of people in an evergrowing number of countries are becoming interested in wine, the drink has taken on a new role. Wine itself is becoming a source of encounters with new people and new experiences. Over the centuries, wine came to be seen not just as a luxury item, but a medicine as well. Many publications described wine as the „most hygienic drink''. Enjoing wine regulary, responsably, and in moderation can contribute to along and healthy life. [1]

Keywords:*fungi, wine, contamination, Penicillium chrysogenum, Penicllium expansum, Phanerochaete chrysosporium, incubated shaker*

1. Introduction

Sometimes, bottles of wine become contaminated by the cork that has been used to seal the bottle. Such bottles are called corked wines because the cork is the most likely source of the taint.

If the contamination is not very significant, the wine usually exhibits a „flat'' odor and may just not taste like a good bottle of the same wine. [2]

The purpose of this paper is to follow the red wine contamination with three different types of fungi. The types are: H.D.P. Roşca, Dry Merlot, Dry Cabernet Sauvignon and Dry Syrah.

Fungi were isolated from samples obtained from contaminated cork (corkwood) stoppers. Three types of isolated fungi were used: *Penicillim chrysogenum, Penicillium expansum, Phanerochaete chrysosporium.*

The identification of the above fungi were made on the basics of their macroscopic (cultural and morphological characters on agar medium) *(Figure 1)* and microscopic structures: the formed of individual conidia the chains of conidia, the structures and types of conidiophores.

2.Material and methods

2.1 Materials

Four types of wine were used for experiment development: (i) H.P.D. Roşca, (ii) Dry Merlot, (iii) Dry Cabernet Sauvignon, (iiii) Dry Syrah.

2.2. Metods

Each type of wine was divided in four Erlenmayers glasses, obtaining sixteen samples. Four samples were preserved intact and the remaining samples were inoculated with the three types of moulds. Then the glasses were agitated for three weks, at 25ºC and 150 rotations per minute in incubated shaker. *(Figure 2)*

One of the experimental purpose was to obtain from each type of wine a healthy sample, the second with *Penicillium chrysogenum*, the third with *Penicillium expansium* and the fourth with *Phanerochaete chrysosporium*.

Microorganisms were isolated from damaged cork samples, plated fragments into PDA plates (potatoes dextrose agar).

After 4 days of incubation at 25ºC, individual fungal colonies were sub-cultured under the same condition, and pure cultures were obtained. Microscopic identification of each the type of fungi was performed. The next analysis were conduct for wine samples:

a) Total Acidity

Regarding to our country, ,,*The Law of Wine and Vinery"* provide as a value for total acidity, in red wines, to be frame between 3 and 4 -5 g/l acid sulphuric .

The total acidity represents the amount of titrable acids, when the wine is brought to the value of pH at 7, by adding an alkaline solution.

The method consist in neutralizing the acidity with a solution of NaOH 0.1 N, using the blue bromothymol as an indicator. Acidity was expressed as sulphuric acid g/l.

As more the wine has a higher acidity, the colour appear being more intense. [3]

b) Total SO_2

The SO_2 is the main substance admitted by ,,*The Law of Wine and Vinery"* as a substance wich preserve the antiseptic and antioxidant quality.

The method consists in titration the sample to be analyzed with solutions of NaOH and H_2SO_4 and the iodomoetric titration of the released sulfure dioxid from its combination structures with sugar and aldehydes in acidic medium. The results were expressed as SO_2 mg / l. [3]

3) Ash

The ash represents the incinerated compounds of total solids. The ash represents 10% from total solids, with variation framed between 1.3- 4 g/l.

As a result of wine maceration technology process, the red wine are richer in ash than the white wine.

The method consists in evaporating the wine using the water bath and calcination electric furnace at temperature range of 450-550ºC. [3]

3. Results and discussions

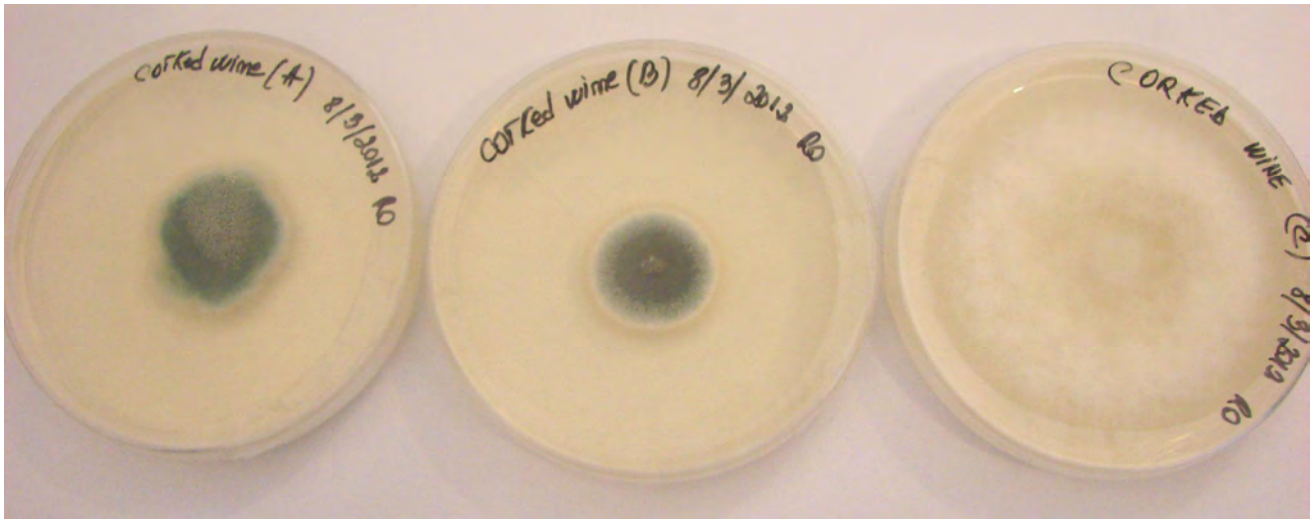
Three types of fungus were identified on the 12 analysed wine samples: *Penicillium chrysogenum, Penicillium expansum* and *Phanerochaete chrysosporium.*

According to *Figures 3 - 4* the healthy wine is within the normal values, *Figure 5* presents an increase value of ash. The color of the wines changes in taint.

Regarding the value of total acidity (*Figure 3*) for all types of wines is maximum 4.07 g/L, so this means that the wine was not contaminated, the wine is not acidulated.

The value of total SO_2 should be framed between 150 – 300 mg/l. According to the *Figure 4* the quantity of total SO_2 for all types of wines is maximum 89.6 mg/l, so the wines were not damaged.

The amount of ash for healthy wine should be framed in concentration range 1.3 to 4 g/l. According to the *Figure 5* the quantity of ash for healthy wines raise the value to maximum 11 g/L, this means that the microorganisms metabolised the total solids.



A)Penicillium chrysogenum B). Penicillium expansium C). Phanerochaete chrysosporium.

Figure 1. Fungi cultures în Petri dishes

Figure 2. Wine contamination process

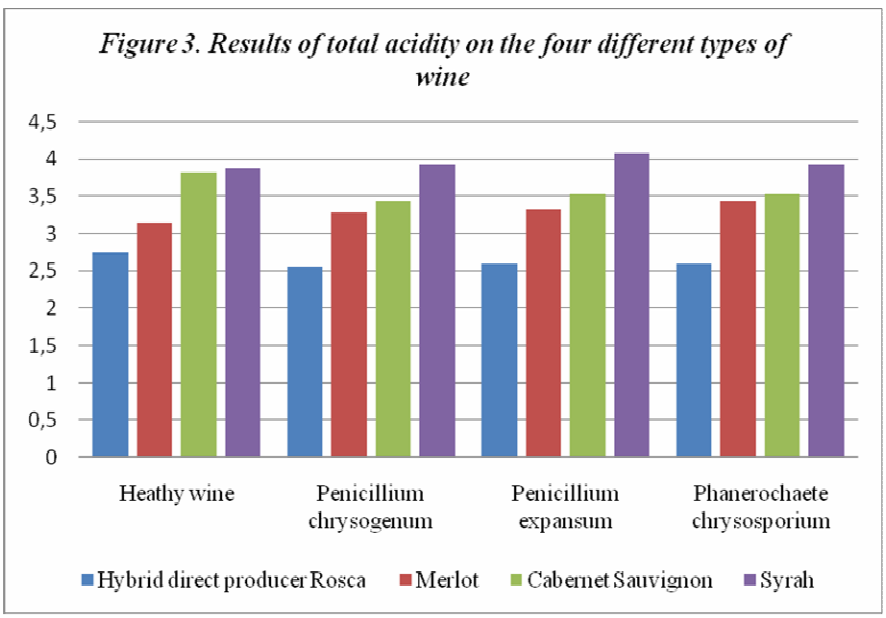


Figure 4. Results of total SO2 on the four different types of wine

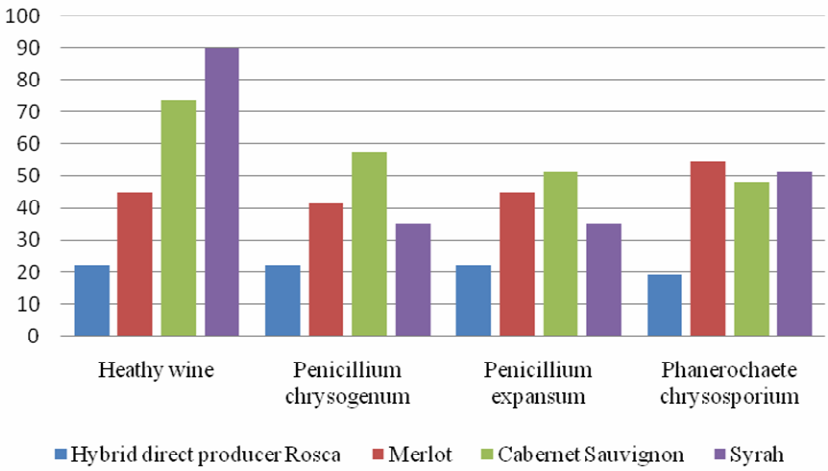
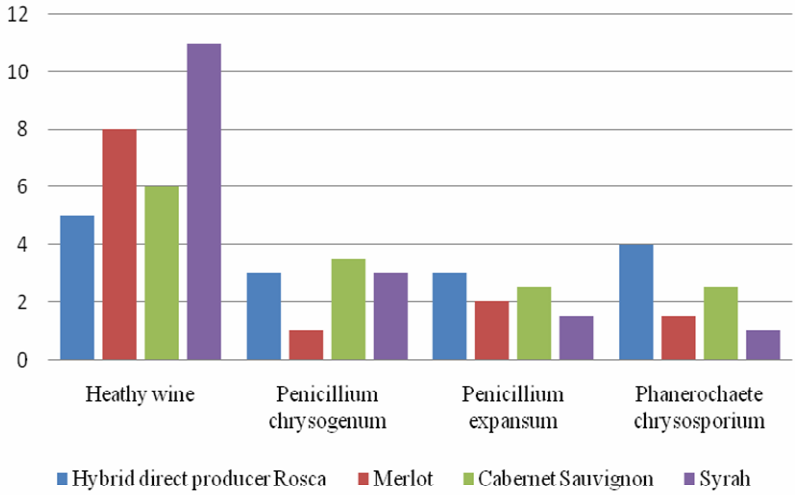


Figure 5. Results of ash on the four different type of wine



4. Conclusions

Concluding the above experiment, we can observe that not every type of fungus can affect or contaminate the wine. In the same time, even if the three fungi had incresed or decreased some values of the wine parameters, especially the colour, taste and acidity, this doesn't mean that the wine is fully damaged, but is not proper for costumers to drink it.

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EFFICIENCY OF THE NANO-PACKAGES BASED ON AG-TIO$_2$ IN PRESERVING THE FRESH CHEESE FROM COW MILK AND YOGURT

Alexandra Mare[1*], Ioana Bob[1]

[1] *Technical University of Cluj Napoca, North University Center of Baia Mare, Department of Chemistry and Biology, Victor Babes 62A, 430083, Romania, phone: 004-0744-79030, peteranca@yahoo.com*

ABSTRACT

Nano-packages containing Ag-TiO_2 supported on polyethylene film were prepared and their preserving activity on fresh cheese from cow milk and yogurt was tested. The food samples were packaged in the nano-packages containing Ag-TiO_2 and were kept at room temperarure in natural light to allow the excitability of the composite. The preserving activity of the nano-packages is the result of the photocatalytic activity of the composite. At different time range, the following physico-chemical parameters of the food samples were monitored: acidity, protein, dry mass and salt contents. Addittionally, the changes of the organoleptic characteristcs were evaluated. The Ag-TiO_2 composites were prepared via sol-gel method, by hydrolysis of tetraisopropoxide and immersing the TiO_2 gels in the solution containing the silver ions. Morphology of the composite was determined by TEM microscopy. The results showed that the nano-packages based on Ag-TiO_2 are more efficient in preserving the investigated food samples during 17 days of storage at room temperature than the conventional polymeric packages.

Keywords: *Ag-TiO_2, nano-packages, fresh cheese from cow milk, yogurt*

1. Introduction

„Nanotechnology" is a colectiv term that defines the technological developments at nanometric scale [1]. Nanomaterials offer a wild domain of applications such as: depolution, syntheses, in all the industry areas, including food domain [1]. TiO_2 is considered one of the most studied semiconducting nanomaterial, for photocatalytic applications due to its chemical stability, non-toxicity, biocompatibility, low cost and easy procurring. Even so, TiO_2 is excitable by UV radiation only, representing 5% from the spectrum of the solar light. An efficient method to extend the light domain that excite the TiO_2 is doping with tranzitional metal particles such as: Ag, Au, Cu, Fe, Co, etc...[2].

In the recent literature, TiO_2 is intense studied in the food industry, for nano-packages preparation, as nano-sensors for monitoring the food safety and as food additives and ingredients [3-18]. Additionally, the preservation and antibacterial activity of TiO_2 is the focus of many recent studies [4, 16-18]. The bottles made of monolayer or multilayer pigmented co-extruded HDPE (High Density PolyEthylene) ensure the preservation of the properties of pasteurized milk for up to five days of storage [16-18]. Li et al. [4] studied the effect of a packaging containing nano-TiO_2 on the capacity of Chinese jujube fruits to preserve their qualities during storage at room temperature. They concluded that these nano-packaging materials could be used to extend the shelf life and to improve the preservation capacity of Chinese jujube.

The aim of this study is to establish if the Ag-TiO_2 based nano-packages can be used as efficient alternative in preserving the quality of the fresh cheese from cow milk and yogurt.

The originality of the paper consists in testing the preserving activity of the Ag-TiO_2, because in the literature, other researches aimed to study the preserving activity of Ag-TiO_2 doesn`t exist.

2. Materials and methods

2.1 Cheese from fresh cow milk and yogurt

The fresh cheese from cow milk was homemade, according to a traditional Romanian recipe. The fresh cow milk (3 l) was allowed to age in air, at room temperature, for 3 days, when the lactic fermentation occurred. The maturated cow milk was then boiled at about 100^0C, for 20 minutes, until all the lactose precipitated. The mixture thus obtained was filtered through a white and clean piece of textile clothing. The fresh cheese from cow milk (1 kg ± 100 g) is the solid phase obtained as a result of the filtering operation.

The yogurt with a fat content of 4.1% was bought in Kaufland`s supermarket in Baia Mare.

2.2 Preparation and characterisation of the Ag-TiO_2 composite. Packages preparation

First the TiO_2 gels were prepared via sol-gel method, by acidic hydrolysis of titanium tetra-izopropoxide (TIP). The molar ratios of the reactants was: [IPT] : [H_2O] : [C_2H_5OH] : [HNO_3] = 1 : 3,325 : 19 : 0.08.

The transparent gel was allowed to age 4 weeks to finallize to polimerization and condensation reactions and then is immersed in solution containing Ag ions (0.005M), for 24 h. Then the gels were washed with ultrapure water, to eliminate the Ag ions in exces. Then, the gels were dried for 24 h, at 80^0C and calcined at 500^0C, 2h, the heat rate being 4^0C/min. The obtained composite was used to the packages preparation.

A Jeol JEM1010 (Japan) Transmission Electronic Microscope operating at an accelerating voltage of 80 kV, coupled with an images acquisition system MegaView III CCD Camera (1.2Mp) Soft Imaging Solutions (Olympus) was employed to obtain TEM images.

The concentration of Ag in the composites was established using the Atomic Absorption Spectrometer AAS 800 Perkin Elmer was 0.21 ± 0.01%.

A suspension of crystallized Ag-TiO_2 (0,05 g) in ethanol (2 ml) was prepared and coated on 400 cm^2 polyethylene film (F-Ag-TiO_2) (Supermarket line Kaufland Romania), using a knife, and dried in air for 10 minutes. Packages, in which, a second polyethylene film was deposited onto Ag-TiO_2 (F-Ag-TiO_2-F) were also prepared.

In the as obtained packages, 50 g food sample (fresh cheese from cow milk and yogurt, respectively) was measured and packed. In the packages with the structure polyethylene-Ag-TiO_2 the food sample is not in contact with Ag-TiO_2 and in the packages with the structure polyethylene-Ag-TiO_2-polyethylene the food sample is not in contact with Ag-TiO_2. For reference, packages consisting in polyethylene film (F) were used in the experiments.

The bags were maintained, at $22,5^0$C, for allowing the visible light to excite the Ag-TiO_2 particles.

At different periods of time (up to 17 days), dry mass, acidity and salt and protein content were determined. Additionally, organoleptic analyses consisted in establishing the variations of color, flavour, aspect and consistence of the food samples deposited in the studied packages, at different time ranges, were performed.

2.3. Acidity measurements

A quantity of 10 g cheese was mixed with ultra pure water (5 ml) and 1 ml phenolphthalein solution 1% (S.C. Chemical Company S.A. Iasi, Romania) was added. The mixture was titrated with solution 0.1N NaOH (S.C. Chemical Company S.A. Iasi, Romania), until the pink colour of the solution persisted for at least one minute. The acidity was calculated using the formula [19](STAS 6353-61):

Acidity (acidity degrees) = V x 10 (1)

where: *V* – volume of NaOH 0.1 N solution used to titration (ml), *10* – sample mass (g)

A quantity of 5 ml yogurt was mixed with ultra pure water (25 ml) and 3-5 drops of phenolphthalein solution 1% (S.C. Chemical Company S.A. Iasi, Romania) was added. The mixture was titrated with solution 0.1N NaOH (S.C. Chemical Company S.A. Iasi, Romania), until the pink colour of the solution persisted for at least one minute. The acidity was calculated using the formula:

Acidity (acidity degrees) = V x 20 (2)

where: *V* – volume of NaOH 0.1 N solution used to titration (ml)

2.4. Salt content

A sample of 5 g was mixed with 15 ml acetone and then 50 ml of ultrapure and a small quantity of sodium carbonate were added. The mixture was filtered and the entirely quantity of extract was added to 0.5 ml of potassium chromate 10% (S.C. Chemical Company S.A. Iaşi, România) were added. The mixture was titrated with 2.906% solution of silver nitrate (S.C. Chemical Company S.A. Iaşi, România) until the color changed in red-brown [19] (STAS 6354-61).

The salt content was calculated using the formula:

$$\%NaCl = \frac{V}{m} \quad (3)$$

where: V – volume of $AgNO_3$ solution used to titration (ml).
m – sample mass (g)

2.5. Protein content

In an Erlenmeyer vessel, 50 g of sample are mixed with 2 ml potassium oxalate solution 2%. Then, 1 ml phenolphthalein 2% was added. The mixture was titrated with NaOH solution 0.143 N until the pink colour appeared, for the free acidity neutralization.

Then, 10 mL formaldehyde solution was added and the mixture was stirred. The pink colour disappeared. The mixture is let to rest for 30 seconds and then is titrated again with NaOH until the pink color appeared [19] (STAS 6355-61). The protein content is calculted as follows:

$$\text{protein } (\%) = \frac{V}{2} \quad (4)$$

where: V- volume of NaOH 0,143 N (ml) used to the second titration

2.6. Dry mass

A sample of 10 g (m_0) was mixed with calcined cuartz sand and the mixture is weight (m_1). The mixture was introduced in the Binder oven at 50...60°C, for 2 h and then at 102...105°C, until the weigh is constant [19] (STAS 6344-61). After cooling the mixture is weigh again (m_2). The percentage of dry mass is calculted with the formula:

$$\text{dry mass}(\%) \frac{m_2 - m_0}{m_1 - m_0} 100 \quad (5)$$

where : m_1 - mass of food sample and sand before drying (g),

m_2 - mass of food sample and sand after drying (g)

m_0 - mass of food sample (g)

All the physico-chemical analyses were repeated three times and the standard deviation was calculated using the Microsoft excel software.

3. Results and disscussion

3.1. Morphological characterisation of the Ag-TiO_2 composite

TEM micrograph (Figure 1) indicates the heterogeneous structure of the Ag-TiO_2 composite. The TiO_2 particles are homogeneously dispersed, but the Ag particles, in some areas, are agglomerated in clusters and in other zones are isolated.

3.2. Organoleptic characterisation of the fresh cheese from cow milk deposited in nano-packages

In Table 1 are presented the organoleptic characterisation of the reference cheese.

After 6 days of storage, the samples packaged in all the investigated packages present a poor altered smell, a little pronounced in the case of F-Ag-TiO_2. The cheese packaged in polyethylene became yellow as compared wih that deposited in the packages with Ag-TiO_2 that remained white, similar with that of the reference.

The aspect of the cheese in polyethylene became mucilaginous at surface, after 6 days of storage, as compared with the samples packages in F-Ag-TiO_2-F and F- Ag-TiO_2 that preserved the normal aspect similar with the reference cheese. No microorganisms spots were observed. The consistence of the cheese in F and F-Ag-TiO_2 was a little sticky, as compared with that packaged in F-Ag-TiO_2-F, that present similar consistence with that of the reference.

After 17 days of storage, the colour of all the samples became yellow. The colour of the cheese deposited in F and F-Ag-TiO_2 became dark yellow, as compared with that of the cheese kept in F-Ag-TiO_2-F which became yellowish. The cheese kept in F and F-Ag-TiO_2 became mucilaginous, but that deposited in F-Ag-TiO_2-F remained granular, having a similar aspect with that of reference (Figure 2).

3.2. Organoleptic characterisation of the yogurt deposited in nano-packages

The organoleptic characteristics of the reference yogurt are: white colour, semifluid creamy consistence, homogeneously aspect, nice smell.

The organoleptic characteristics of the yogurt deposited in the three packages, after 6 days of storage are presented in Table 2. No dark spots of microorganisms were observed.

The organoleptic characteristics of the yogurt deposited in the three packages, after 10 days of storage are presented in Table 3.

The organoleptic characteristics of the yogurt deposited in the three packages, after 17 days of storage are presented in Table 4.

The image of the yogurt samples kept in the three packages for 17 days are also presented in Figure 3.

The most efficient package for preserving the organoleptic characteristics of the yogurt is F-Ag-TiO_2-F.

3.3.Physico-chemical analyses of the samples deposited in nano-packages

In Table 5 are presented the physico-chemical parameters of the food samples kept in different packages.

The acidity of the fresh cheese from the cow milk packaged in all the investigated packages decreased. The lower decrease was observed in the polyethylene and the most accentuated in the F-Ag-TiO_2 package due to the photocatalytic activity of Ag-TiO_2 that, on one side is a good barrier for oxygen, reducing the oxidation rate and on the other side, decompose de acidic compounds generated in the lactic fermentation process [3].

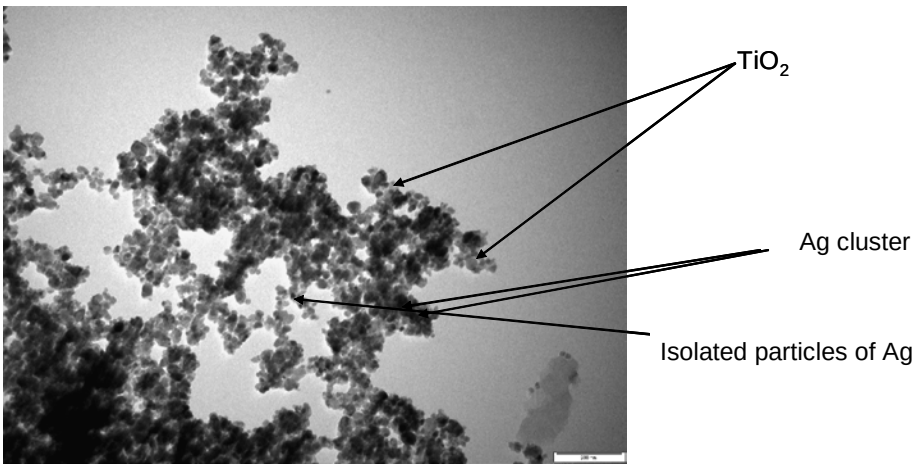

Figure 1. TEM micrograph of the Ag-TiO_2 composite

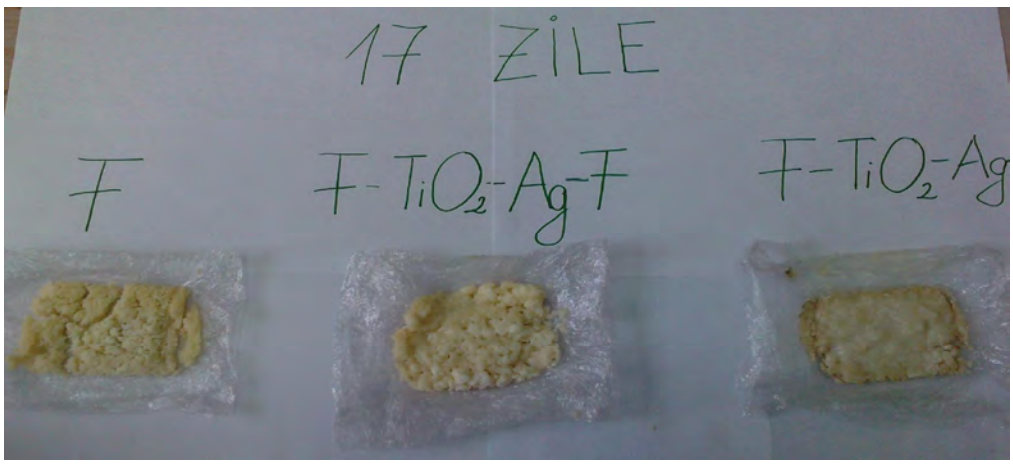

Figure 2. Image of the cheese deposited in the three packages after 17 days of storage (F-polyethylene)

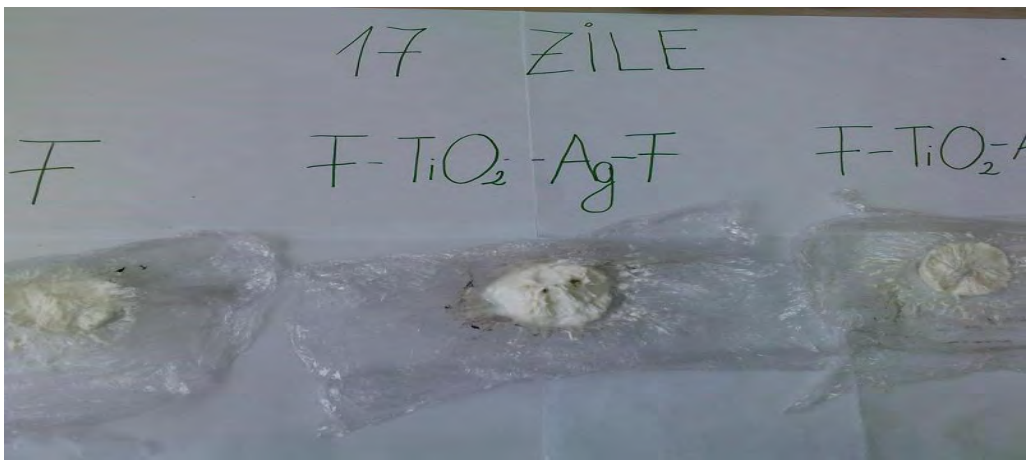

Figure 3. Image of the yogurt kept in the three packages for 17 days (F-polyethylene)

Table 1. *Organoleptic examination of the cheese at 0 days*

Parameter	Characterisation
Colour	White, uniformous
Aspect	Homogeneous, whitout whey drain
Consistence	Homogeneous, rough, creamy structure
Flavour	Nice

Table 2. *Organoleptic characteristics of the yogurt deposited in the three packages, after 6 days of storage (F-polyethylene)*

	Package		
Parameter	**F**	**F-TiO_2-Ag-F**	**F-TiO_2-Ag**
Colour	White mat	White mat	White mat
Aspect	Semifluid	Fluid	Semifluid
Consistence	Similar with the reference	Similar with the reference	Similar with the reference
Flavour	Normal, nice	Normal, nice	Normal, nice

Table 3. *Organoleptic characteristics of the yogurt deposited in the three packages, after 10 days of storage (F-polyethylene)*

	Package		
Parameter	**F**	**F-TiO_2-Ag-F**	**F-TiO_2-Ag**
Colour	White in bulk, yellowish at surface	White mat	White mat
Aspect	Mold spots at surface	Normal	Mold spots at surface
Consistence	Sticky	Similar with the reference	Sticky
Flavour	Altered	Little altered	Altered

Table 4. *Organoleptic characteristics of the yogurt deposited in the three packages, after 17 days of storage (F-polyethylene)*

	Package		
Parameter	**F**	**F-TiO_2-Ag-F**	**F-TiO_2-Ag**
Colour	Yellowish	White mat	Yellowish
Aspect	Mold at surface	Little fluid	Mold at surface
Consistence	Sticky	Liquid	Coagulated
Flavour	Deeply altered	Little altered	Deeply altered

Table 5. *Physico-chemical parameters of the food samples kept in different packages (F-polyethylene, SD – standard deviation)*

Range	0 day				6 days				17 days			
Cheese from fresh cow milk												
Packa-ge ID	**salt [%] ±SD**	**Acidi -ty [^{0}T] ±SD**	**Dry mass [%] ±SD**	**Protein [%] ±SD**	**salt [%] ±SD**	**Acidi -ty [^{0}T] ±SD**	**Dry mass [%] ±SD**	**Protein [%] ±SD**	**salt [%] ±SD**	**Acidi -ty [^{0}T] ±SD**	**Dry mass [%] ±SD**	**Protein [%] ±SD**
F	0.8 ±0.03	168.1 ±0.28	25.79 ±0.36	5.36 ±0.05	0.63 ±0.01	145 ±0.01	35.66 ±0.45	3.91 ±0 .02	0.54 ±0.05	102.3 ±2.3	44.5 ±0.29	2.6 ±0.01
F-Ag-TiO_2-F					0.59 ±0.01	110 ±0.01	30.46 ±0.42	3.62 ±0.32	0.43 ±0.01	61.6 ±2.51	61.6 ±0.35	3.11 ±0.33
F-Ag-TiO_2					0.49 ±0.01	64.5 ±0.02	33.28 ±0.35	5.02 ±0.01	0.20 ±0.04	47 ±7	47 ±0.44	4.36 ±0.1
Yogurt												
F	1.67 ±0.01	95.0 ±1	13.16 ±0.01	3.5 ±0.03	1.16 ±0.01	112 ±0.01	17.85 ±0.01	2.18 ±0.02	0.65 ±0.02	124 ±1.15	20.23 ±0.05	1.85 ±0.05
F-Ag-TiO_2-F					1.23 ±0.01	104. ±0.01	15.65 ±0.05	2.28 ±0.02	0.68 ±0.01	118.6 ±2	18.75 ±0.05	1.95 ±0.01
F-Ag-TiO_2					1.27 ±0.01	99.3 ±4.16	16.62 ±0.01	2.9 ±0.001	0.67 ±0.02	117.6 ±2.08	19.25 ±0.05	2.41 ±0.07

The acidity of the yogurt kept in polyethylene increases during 17 days of storage, due to the generation of the acidic compounds in the lactic fermentation. The acidity of the yogurt kept in packages with Ag-TiO_2 is lower than that of the sample kept in F, due to the photocatalytic activity of Ag-TiO_2 [3].

The salt content of the cheese decreases in all the three packages. The most accentuated decrease was observed in cheese kept in F- Ag-TiO_2, from 0,8% to 0,2% after 17 days of storage.

After 6 days of storage, the sample deposited in F-Ag-TiO_2 presents a salt content of 0,49%, but after 4 more days, the salt content decreases to 0,35%.

The salt content of the samples kept in F, after 6 days, is 0.63%. The value is with 0.17% lower than the salt of the reference. After 17 days, the salt content of the cheese kept in F decreases to 0.54%.

The salt content of the cheese in F-Ag-TiO_2-F is 0,. 9% after 6 days. The salt content continuously decreases to 0.43%, after 17 days.

The salt content of the yogurt samples decreases during storage, in all the three packages. The more accentuated decrease was observed in F, as compared to that in the packages containing Ag-TiO_2.

The decrease of the salt content is explained by the chemical processes occuring at the TiO_2`surface, when the chloride ions are oxidized by the photogenerated coupled and form molecular chlor that leaves the system (reactions 1 and 2) [3].

$$h^+ + Cl^- \rightarrow Cl\cdot \quad (1)$$

$$Cl\cdot + Cl\cdot \rightarrow Cl_2\uparrow \quad (2)$$

The dry mass content of the cheese samples increases during 17 days of storage in all the investigated samples. The highest increase was observed for the cheese kept in F-Ag-TiO_2-F (35.81%), as compared with the variation in F-Ag-TiO_2 (21.2%) and in F (18.8%). The increase of the dry mass ii was previously reported in the literature [4, 5, 15, 16] and is explained by the low permeability of the TiO2 for oxygen, thus diminishing the oxidation processes, that generates water.

The dry mass of the yogurt also increased during storage. The highest variation occurs in F package.

The protein content of the cheese and yogurt decreases during storage, after 17 days. The most accentuated decrease of the cheese and yogurt was observed in F package and the most reduced in the F-Ag-TiO_2 package, thus demonstrating the efficiency of the Ag-TiO_2 in preserving the protein content of the cheese and yogurt. This behaviour is explained by the low permeability for oxygen of the TiO_2, that reduces the degradation rate of the proteins and aminoacids, as compared with the case that occurs in F package, when, the proteolysis processes occur.

4. Conclusions

The most accentuated decrease of the acidity, salt content and dry mass and the most reduced decrease of the protein content was observed for the cheese and yogurt samples deposited in Ag-TiO_2 containing packages.

The most efficient packages for preserving the organoleptic and physico-chemical properties of fresh cheese from cow milk and yogurt is consisting in a „sandwich" structure of polyethylene- Ag-TiO_2 - polyethylene film.

5. Acknowledgement

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POTENTIAL ANTIFUNGAL ACTIVITY OF OLIVE MILL WASTEWATER AGAINST POSTHARVEST DISEASES OF PEARS

Ioannis Vagelas[1*], Nikolaos Gougoulias[1], Liviu Giurgulescu[2], Ioanna Papageorgiou[1]

[1]*Technological Educational Institute of Larissa, Department of Plant Production, 41110 Larissa, Greece, vagelas@teilar.gr*

[2] *Technical University of Cluj Napoca, North University Center of Baia Mare, Department of Chemistry and Biology, Victor Babes 62A, 430083, Romania*

ABSTRACT

The antifungal activity of olive oil mill wastewater (olive OMW) was investigated against common postharvest diseases of pears. The effect of sterilized, filtered sterilized and non sterilized olive OMW was tested a) on mycelium growth of postharvest diseases caused by Botrytis cinerea, Penicillium expansum and Alternaria alternata in vitro and b) on pears fruits in vivo infected with the fungus P. expansum. The results show that the filtered sterilized olive OMW inhibits the growth mycelium of the tested molds in vitro. Heavy metals such as Cu and Zn and high values of total phenolic compounds found on olive OMW suggested the antifungal activity of the olive waste water solution.

Keywords: *Biocontrol, Gray Mold, Blue Mold, Botrytis cinerea, Penicillium expansum, Alternaria alternata*

1. Introduction

During olive oil extraction a large amount of solid and aqueous residues known as olive oil mill wastewaters (olive OMWs) are produced annually worldwide where the majority of it is produced in the Mediterranean basin. The uncontrolled disposal of olive OMW is becoming a serious environmental problem due to its high content in phenolic compounds: tannins and flavonoids [1, 2]. Some of these phenols are responsible for several biological effects, including antibiosis [3] (Rodrvguez *et al.*, 1988) and phytotoxicity [4]. They also appear to be involved in the defense of plants against invading pathogens, including bacteria, fungi and viruses [5]. The use of olive OMW for plant and harvested fruits protection against microorganism could be a solution for residues management and nature protection. The main objective on this study was to examine the biological control of filtered sterilized olive OMW against common postharvest diseases of pears caused by *Botrytis cinerea, Penicillium expansum* and *Alternaria alternata*.

2. Material and methods

2.1. Isolation and characterization of fungal organisms involved in the experiment

Botrytis cinerea, Penicillium expansum and *Alternaria alternata* isolated from infected pear fruits were used for this experiment. Pathogens were cultured on potato dextrose agar (PDA; DIFCO) at 25 ^{o}C. The identification of fungi was made based to CAB international descriptions of fungi and bacteria.

2.2. In vitro assessment of antimicrobial activity of olive OMW against fungi mycelia

The antifungal effect of olive OMW against *B. cinerea, P. expansum* and *A. alternata*, mycelia was tested *in vitro*. Tests

were made on PDA in 9 cm Petri dishes. Treatments were PDA plates with a) olive OMW added into the medium and autoclaved and b) a drop of filter sterilized olive OMW (using a syringe Millipore filter 0.2 μm) added onto the agar surface (treatment b). These are fully described in Vagelas *et al.*, (2009a). In the first treatment (treatment a), a 25ml of olive OMW were added into 1l agar and further sterilized by autoclaving (121 ^{0}C for 20 min). In the second treatment, olive OMW was filtered through four layers of sterile cheesecloth in order to remove the solid phase material, passed through Whatman filter paper No 2, then whole solution was passed through whatman filter paper No 4 before filtered further using a syringe Millipore filter 0.2 μm and finally a drop (50 μl) of filter sterilized olive OMW liquid solution (Fig. 1A; OMW), was added onto the centre of each plate. Fifteen agar plates per treatment were inoculated with a mycelium plug (5 mm in diameter) of the above fungi which was taken from the periphery of 7 days old fungal colonies. Mycelia plugs were placed onto the centre of each plate or next to the olive OMW drop. Equal plate numbers were used as control (without olive OMW). Plates were incubated at 25^{o}C for six days and fungus mycelium growth was recorded.

2.3. In vitro assessment of antimicrobial activity of olive OMW against blue mold on pears caused by Penicillium expansum

Fresh market healthy pear fruits were artificial inoculated with spore suspension of *P. expansum*, incubated in plastic box chambers at 10 ^{o}C and assessed mold lesions caused by *P. expansum* using a six level scale of damage. In details, surface sterilized pear fruits were wounded with a needle and inoculated with a drop of 100 μl spore suspension of *P. expansum* in sterilized distilled water or in sterilized olive OMW, containing 0.05% (v/v) Tween 80. Spore suspension of *P. expansum* was prepared by isolating spores of fungus, from 5 days old cultures. Three agar plates of fungus culture were used to collect spores. Spores were collected in 250ml Erlenmeyer flask which contained distilled water by washing the agar surface with 3ml distilled water and filter the produced solution through sterilized muslin. In each flask fungus spores suspension was adjusted, with the aid of a hemocytometer, at a final concentration 10^6 spores/ml (Fig. 1A). Pear fruits were inoculated with that spore suspension of *P. expansum.* Treatments were fruits inoculated a) with a drop 100μl/fruit of spore suspension of *P. expansum*, b) with a drop 100μl/fruit of spore suspension of *P. expansum* mixed with a (10μ*l*) sterilized olive OMW liquid solution (Fig. 1A) at a volume 1/10v:v. Controls were fruits treated only with sterilized olive OMW or treated only with sterilized distilled water (SDW). All treated fruits (25/treatment) were placed in plastic box chambers and kept at 10 ^{o}C for 14 days. The plastic box chambers were spayed every 2nd day with SDW to achieved high humidity conditions (>80%RH). After the incubation period, the lesion diameter of blue mold, caused by *P. expansum*, were recorded and sorted in six level scale of damage (0-5; Fig. 1B), where 0 is equal to healthy fruits, 1= slightly visible lesion, 2= lesions up to 6mm without spores, 3= lesions up to 10mm without spores, 4= lesions up to 16mm with visible spores 5= lesions >20mm heavy infected with *P. expansum* blue spore masses (Fig. 1C).

2.4.Determination of macro-micro elements and phenolics compound in solution

Samples were analysed using the following methods which are referred by Page *et al.* [6]. Organic carbon was analysed by chemical oxidation with 1 mol L^{-1} $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 mol L^{-1} $FeSO_4$.

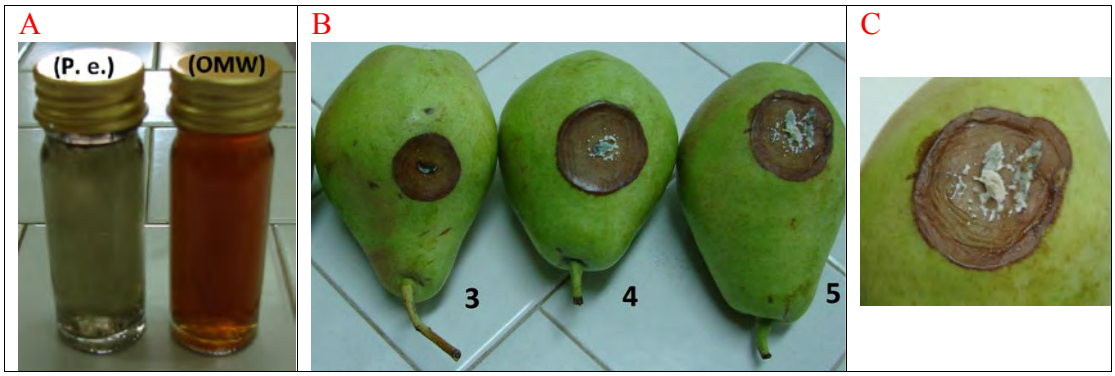

Figure 1. A: Spore suspension of P. expansum (P. e.) and olive OMW liquid solution (OMW). B and C: Symptoms caused by P. expansum on pears. Scale of damage (B), P. expansum blue spore masses (C).

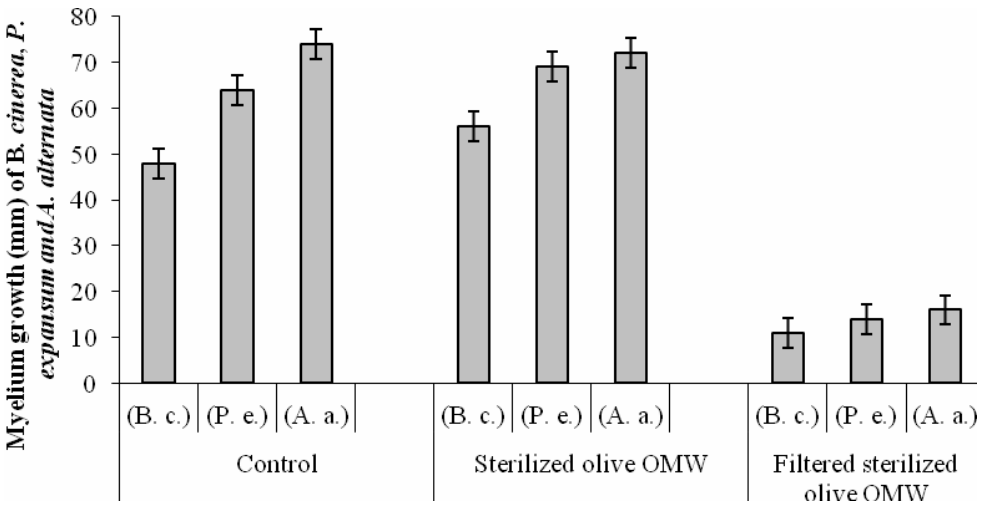

Figure 2. Effect of sterilized and filter sterilized olive oil mill wastewater (olive OMW) on the mycelium growth of B. cinerea (B. c.), P. expansum (P. e.) and A. alternate (A. a).

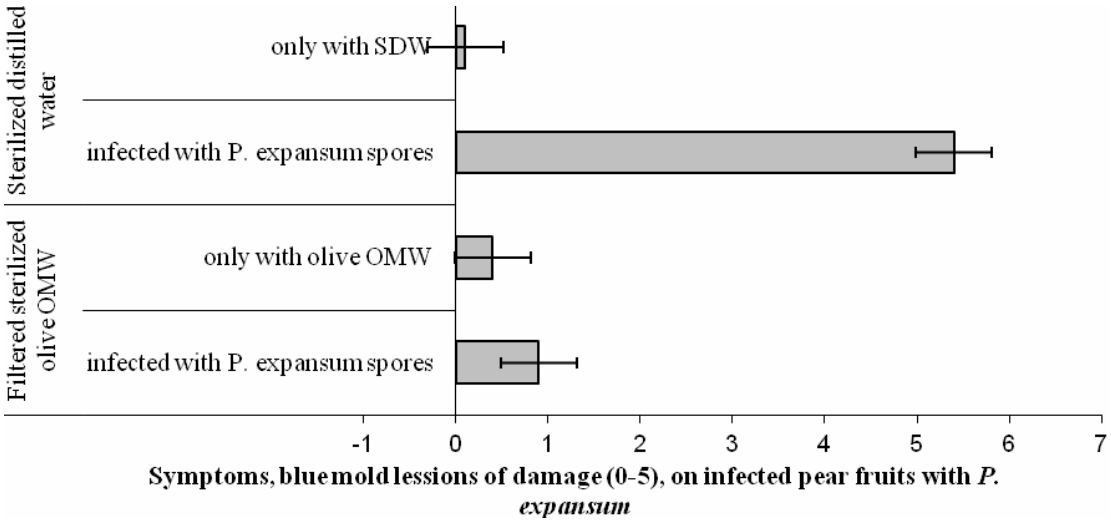

Figure 3. Effect of filter sterilized olive oil mill wastewater (olive OMW) and sterilized distilled water on blue mold symptoms formation of Penicillium expansum on pear fruits in vivo.

Table 1. *Chemical properties of the olive oil mill wastewater*

Parameter	Olive oil mill wastewater properties
Solid concentration of (g . L^{-1}), olive oil mill wastewater	63.8
Electrical conductivity, dS m^{-1}	1.83 (raw waste)
pH	5.10
Organic matter (g kg^{-1})	60
Total N (g kg^{-1})	0.54
Total P (mg kg^{-1})	302
Total K (mg kg^{-1})	3.820
Total Zn (mg kg^{-1})	5.1
Total Cu (mg kg^{-1})	12.7
Total phenols (mg (GAE)./L^{-1})	4.340

The organic carbon contents were transformed in organic matter contents by multiplying by 1.724, which is an experimental factor, reported by Hesse [7]. Total polyphenols (TP) contents were determined with the Folin-Ciocalteu reagent according to the method of Singleton and Rossi [8] and were expressed as gallic acid equivalent (GAE).

2.5.Statistical analysis

Data were analyzed using the Minitab statistical package. Analysis of variance (ANOVA) was used to assess treatments effect. Mean separations were performed using Tukey's multiple range test.

3. Results and discussion

3.1. In vitro assessment of antimicrobial activity of olive OMW against fungi mycelia

There was a statistical significant difference between filtered sterilized olive OMW and control (untreated PDA and sterilized with olive OMW PDA), (P<0.001). Moreover, the filter sterilized olive OMW significantly inhibited the growth of *B. cinerea, P. expansum* and *A. alternata* mycelia *in vitro* showed a biocontrol ability (fungistatic activity, probably due to phenols content), to control mycelia of postharvest diseases of pears caused by *B. cinerea, P. expansum* and *A. alternata* (Fig. 2).

3.2. In vitro assessment of antimicrobial activity of olive OMW against blue mold on pears caused by Penicillium expansum

Filter sterilised olive OMW was effective in controlling blue mold caused by *P. expansum* in pear fruits *in vivo* tests. In details, the filter sterilised olive OMW reduced significantly (P<0.001) the lesion caused by the pathogen and the number of *P. expansum* spores developed on the infected pear fruits compared with those treated only with *P. expansum* spores (Fig. 3).

3.3. Determination of macro-micro elements and phenolics compound in solution

In Table 1, it is observed that main nutrients, N, P and K found in the tested olive oil mill waste water solution. Specifically, highest N (0.54g/kg) concentration was found in the olive oil mill waste water. Further in the Table 1 the Cu (12.7 mg/kg) and Zn (5.1 mg/kg) contents of olive OMW were in high values ranged in the following order: Cu>Zn. This indicates that olive OMW is a source of heavy metals such as Cu and Zn, ions that probably affected the growth of microorganisms. It is well know that metals such as cooper and zinc in a high concentration show toxicity to filamentus fungi belonged to genera *Aspergillus*, *Penicillium* and *Alternaria* [9].

Olive oil mill wastewater (olive OMW) contains phytotoxic components capable of inhibiting the growth of microorganisms [10, 11] and plants. Olive OMW contains phenolic compounds [10] polysaccharides, lipids, proteins, and a number of monocyclic and polymeric aromatic molecules [4] which might exhibit inhibition effects towards some specific microorganism populations. In the current study filter sterilised olive OMW significantly reduced the growth common postharvest diseases of pear caused by *B. cinerea, P. expansum* and *A. alternata*. According to D'Annibale [12] phenolic compounds are the main determinants of the phytotoxic effect of olive residues. Thus, the phenolics of olive OMW used in this experiment had negative effect on tested mycelia fungus pathogens *in vitro*. The used for olive OMW sterilization at 121 ^{0}C for 20 min probably removed or destroyed the phenolic compounds from olive OMW solution showed same results as the untreated (mycelia – controls) *in vitro*.

Furthermore, blue mold symptoms and spores production of *P. expansum* on pear fruits significantly inhibited by sterilized olive OMW *in vivo*. Olive OMW shows high values of heavy metal ions (copper and) zinc) and total phenols recommended the toxicity of the solution to microorganisms. Based on that, we assume that the presence of Cu, Zn and phenolic compounds on olive OMW suppresses fungus reproduction and possible could offer a protection on pears or other fruits such as apples from common postharvest diseases. Overall we believe that the sterilized olive OMW due to phenolics have antifungal activity and could possible used against many other postharvest plant fungal pathogens for preventing common diseases and other mold development.

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ACCUMULATION OF CHLOROPHYLL AND CAROTENOIDS PIGMENTS IN PLANTS GROWN UNDER ARTIFICIAL LIGHT

Camelia Nicula[1*], Alina Buie[1], Oana Bran[1]

[1] Technical University of Cluj Napoca, North University Center of Baia Mare, Department of Chemistry and Biology, Victor Babes 62A, 430083, Romania, phone: 004-0744-79030

*vargacamelia@yahoo.com

ABSTRACT

Curiosity concerning how some environmental factors influence the amount of chlorophyll and carotenoid pigments that accumulate in plants when they grow, was the reason for choosing this work. This paper studies the accumulation of chlorophyll and carotenoid pigments in four types of plants grown under artificial light action: parsley (*Petroselinum crispum*), dill (*Anethum graveolens*), beet (*Beta vulgaris*) and radish (*Raphanus sativus*).

Keywords: chlorophyll, carotenoid pigments, *Petroselinum crispum*, *Anethum graveolens*, *Beta vulgaris*, *Raphanus sativus*.

1. Introduction

The recommendation of chlorophyll and carotenoid pigments is now more diversified and numerous studies being done about it. Chlorophyll and carotene can be extracted from many plants.

The chlorophyll is one of the substances with most pronounced antioxidant character. That in vitro can neutralize the action of oxidants has been demonstrated. The beneficial effects of chlorophyll and carotenoid pigments were found with many years ago. They are particularly important for plants well as humans: helps repair and development of tissues, helps to eliminate toxins, helps the transport of oxygen and magnesium by blood to cells and tissues, stimulates production of red blood cells and helps retain calcium and other minerals.

The vegetables are particularly important in human nutrition. A rational diet is unthinkable without daily use of vegetables in a wide range. Vegetables have a particularly favorable effect on health due to their rich content of vitamins, minerals and other essential components of a good functioning of the the human body. Their content is influenced by species, varieties, climates and applied cultivation technologies.

Thus, light, temperature, humidity and type of soil are just some of the elements that influence plant growth and development of vegetable. A very important thing to remember is that these factors are closely linked to each other, and to get the desired results is necessary to be carefully observed all these parameters for each plant species separately.

All the organisms need nutrients to perform their life. They take these substances from the environment and through nutrition processes transform them in the body's own substances. The green plants take over the water, minerals and CO_2 from the environment and using the light energy converts them in organic substances and O_2. The existing pigments in plants absorb light quanta, thus establishing the essential premise for the process of photosynthesis.

The role of pigments in the photosynthesis was revealed showing that the process takes place only in the green parts of plants and only in presence of light. In the process of adaptation to environmental conditions, the plants have developed a pigments system capable to use the radiation found in abundance in the living environment. In the process of photosynthesis can be used not only radiation absorbed by chlorophyll, but also those absorbed by carotenoids.

Leaf chlorophyll content varies within wide limits (from 0.05 to 0.30% of fresh matter). According to the majority of investigators, the ratio between chlorophylls a and b is 3:1. These values vary as a function of plant growth and development, the cultivar of plant and a number of environmental factors. The greatest chlorophyll content in plants occurs at the outset of the flowering phase and is usually 4-5 mg of chlorophyll per unit of leaf surface [1]. It should also be stressed that color of the leaves of certain cultivars and varieties is not always directly correlated with chlorophyll concentration.

Carotenoids have a very important role in photosynthesis. Biosynthesis of carotenoids in plants is a genetic characteristic, but environmental conditions also have an essential role.

Many authors have established that chlorophyll synthesis is dependent upon mineral nutrition. This significantly affects the dynamics of leaf surface formation and the extend of leaf surface reflected in the total sum of leaf surface which is the photosynthetic potential. Of all macrometabolic elements, the greatest influence on development of plants in general and their leaf surface is exerted by N, whose effect is enhanced by P and to a lesser extent by K.

Nitrogen concentration in green vegetation is related to chlorophyll content, and therefore indirectly to one of the basic plant physiological processes: photosynthesis [2-5]. Nitrogen is an essential element for plant growth and is frequently the major limiting nutrient in most agricultural soils [6]. Phosphorus is involved in many metabolic processes essential for normal growth, such as photosynthesis. This element exert influence on stability of the chlorophyll molecule. Potassium is also essential for photosynthesis because it activates many enzymes involved in this process [7].

The chlorophyll and carotenoid pigments content depended on the presence and ratio of mineral elements in the substrate [8].

Light plays an important role in the development of a plant. Processes such as photosynthesis and phototropism, depend on the availability of light sources for plants [9].

Cultivations carried out under poor illumination conditions present higher biomass chlorophyll content than cultivations carried out under high illumination conditions, suggesting an inverse proportional relationship between light intensity and chlorophyll content [10, 11]. Moreover, the use of high light intensity in cultivation of some species can lead to two main effects: (i) photoinhibition, decreasing the cellular growth rate, and (ii) photooxidation, with severe cell damage and, in extreme cases, total loss of the cultivation [12, 13]. Although photoinhibition usually occurs at light intensities above the saturation of the photosynthetic rate, this phenomenon can be observed at light intensities below the saturation of the photosynthetic rate in cultivations under stress conditions, such as low temperatures [14,15].

This paper studies the accumulation of chlorophyll and carotenoid pigments, the action of artificial light radiation in four species of herbs: parsley (*Petroselinum crispum*), dill (*Anethum graveolens*), beet

(*Beta vulgaris*) and radish (*Raphanus sativus*).

Parsley is a plant resistant to cold, which may hibernate in the field without problems. Seeds germinate at 2°C to 3°C, and plants resistant to fresh east -8°C to 9°C. Has poor requirements from humidity. The largest claims against moisture presents during the seed germination and the roots thickening. Soils that do well are those with mild or medium texture, well loose, permeable, deep, humus 4% to 5%, pH 6.5 to 7.5. Soils fertilized with fresh manure is not recommended.

Dill is a plant resistant to low temperatures. Germination begins at 3°C. Optimum growth temperature is 16°C to 18°C. Requirement is moderate to light, but an insufficient amount of light leads to the elongated and less aromatic plants. Has a high claim to moisture during the seed germination and the first phase of vegetation. Excess moisture does not support. Soil is not a limiting factor, because it grows well on almost all soil types.

Red beets start to germinate at 6°C to 8°C and optimum development temperature is 18°C to 20°C. Plants spring up after 9 to 13 days if conditions are optimal. Young plants die at temperatures below -3°C to -2°C. After the first leaves growing, the plant became more resistant also to the lower temperatures (between -8°C to -5°C). Water should be in large quantities during the germination and root thickening stage. Soil moisture limits should be maintained at 60% to75%. The soil should have a medium texture, high water holding capacity and to be rich in organic matter and nutrients. Light is a key determinant of growth and development of red beet plants but does not support shading.

Radish is grown spring and autumn when the days are shorter. Seed germination temperature is 3°C to 5°C and the older plants resistant at -6°C to -5°C. At temperatures below 6°C to 8°C, plants not growing. Soil moisture must be kept constant at 65% to 75%. Recommended for growing radishes soils are medium textured (clayey and sandy or sandy-loamy), rich in organic matter (3.5%) with optimum pH of 6.

2. Materials and methods

2.1. Planting the seeds

The experiment was conducted during November-December 2011.

In the experiment were used:

- seeds of dill, parsley, beetroot and radish from the SEM-LUCA company,
- SOLVIT-G topsoil, with pH between 6 to 6.7, rich in minerals extracted from natural ingredients: black peat, ground of leaf, ground of celery, river sand.
- plastic pots.

For each type of plant seeds have been sown 300 (3 pots with 100 seeds).

After sowing, the land was well watered, and then pots were placed in vegetation chamber SANYO MLR - 351H.

2.2. Experimental conditions

The temperature, humidity and intensity of artificial light inside the vegetation chamber were set to values shown in Figure 1 (a) - (c).

The vegetation chamber is equipped with 15 lamps that can be started separately, depending on lighting step. Correlation between lighting step and the number of started lamps is the following: step 0 - no lamp started, step 1 - one lamp started, step 2 - 2 lamps started, step 3 - 3 lamps started, step 4 - 9 lamps started, step 5 - 15 lamps started. Correlation between light intensity and light step into the vegetation chamber is shown in Figure 2.

The plants were monitored in time in terms of height and content of chlorophyll and carotenoid pigments accumulated.

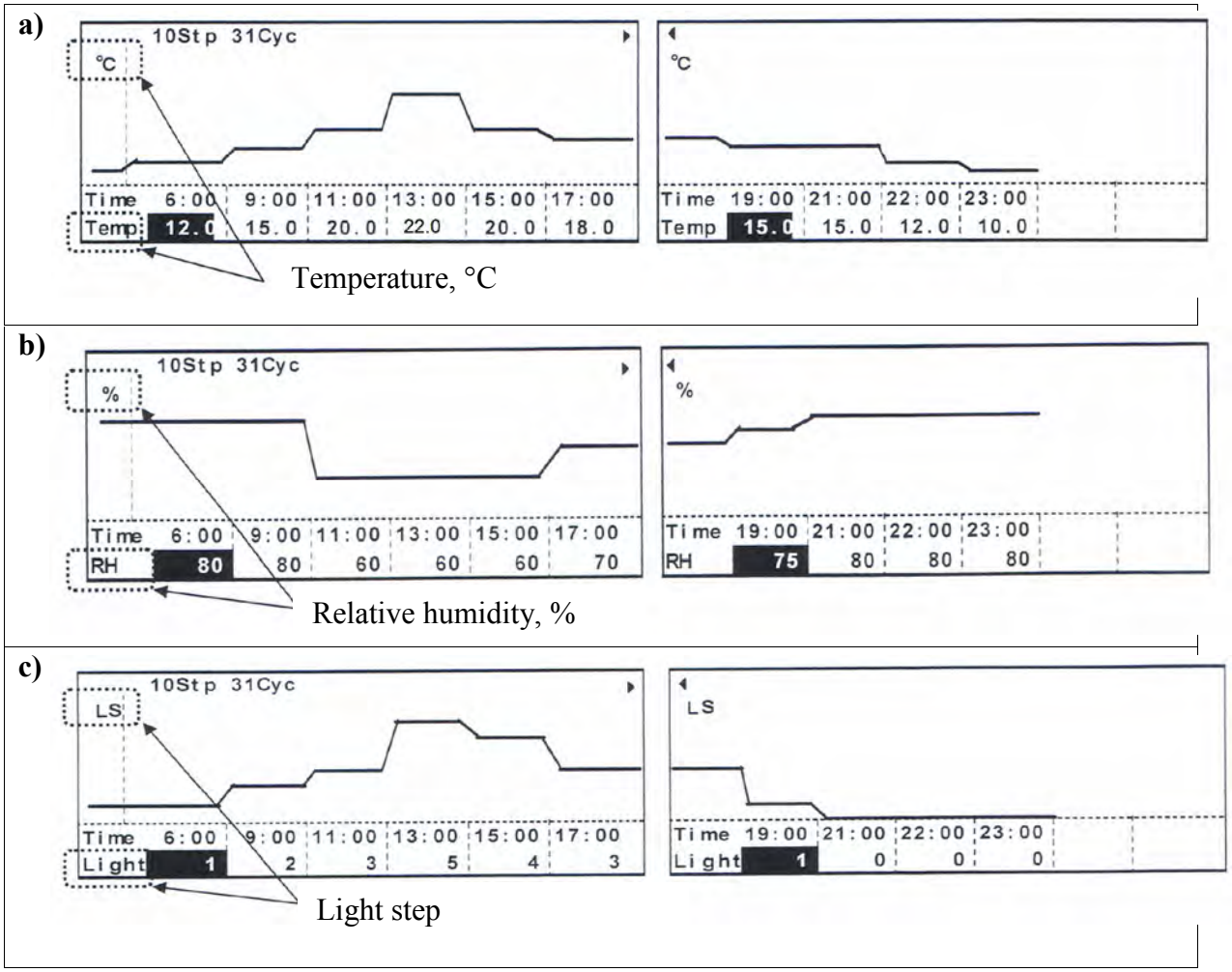

Figure 1. Variation of temperature (a), humidity (b) and light intensity (c) at different times of day, inside the vegetation chamber

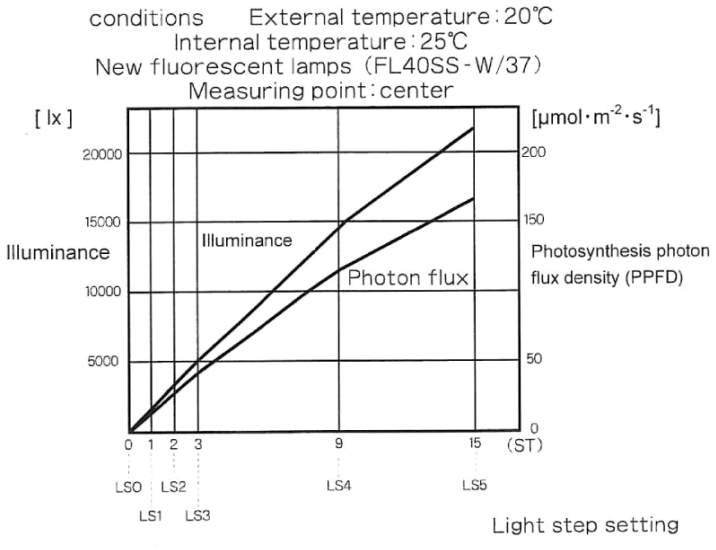

Figure 2. Correlation between light intensity and light step into the vegetation chamber.

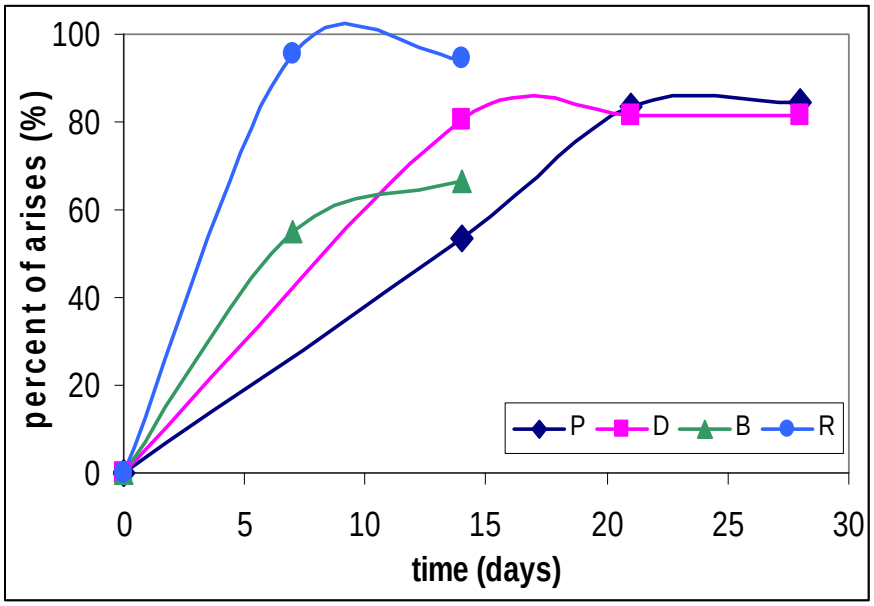

Figure 3. Sprouting percent values at various periods of time from seeding.

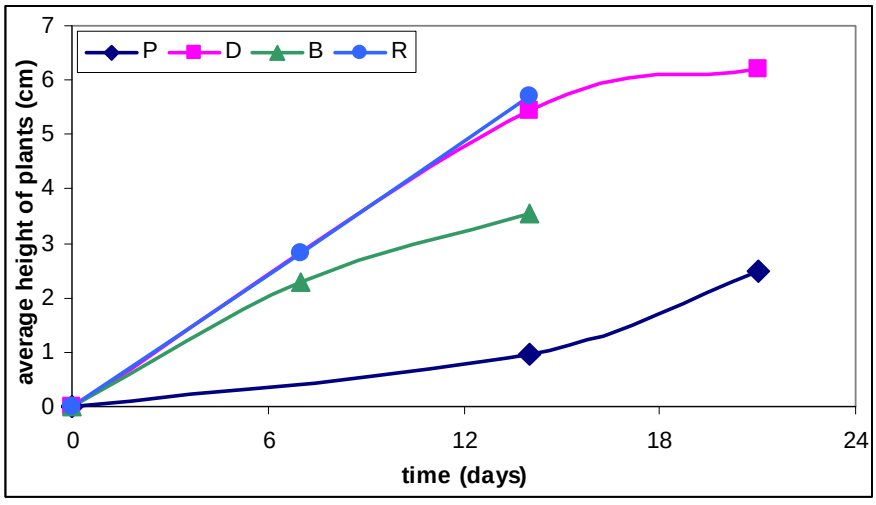

Figure 4. Time variation of the average height of plants

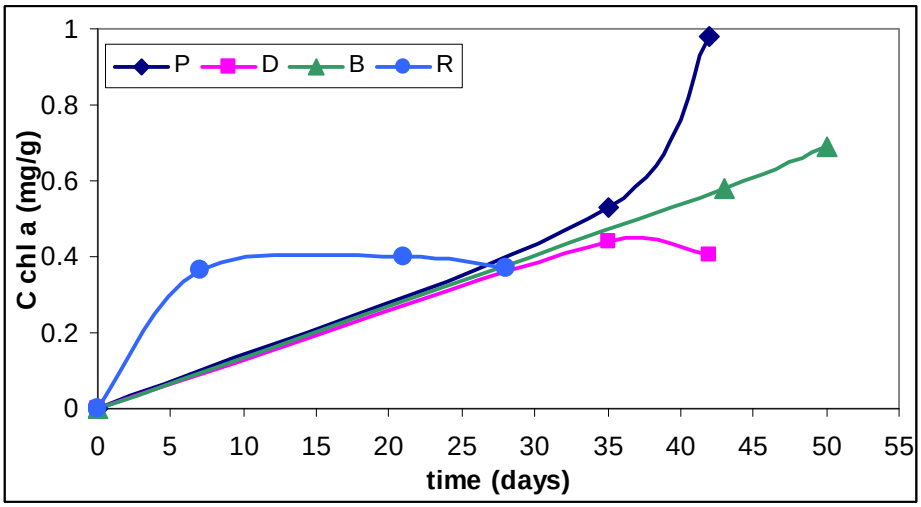

Figure 5. The chlorophyll a accumulation in species analyzed.

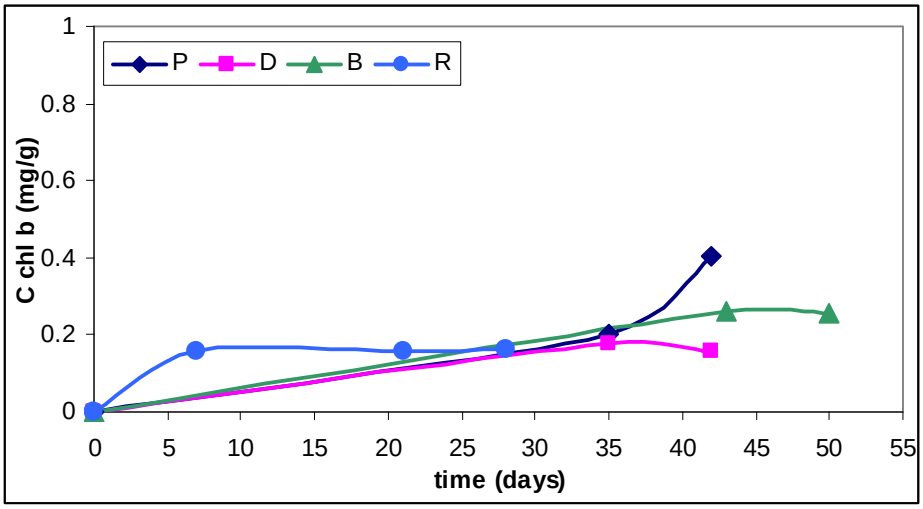

Figure 6. The chlorophyll b accumulation in species analyzed.

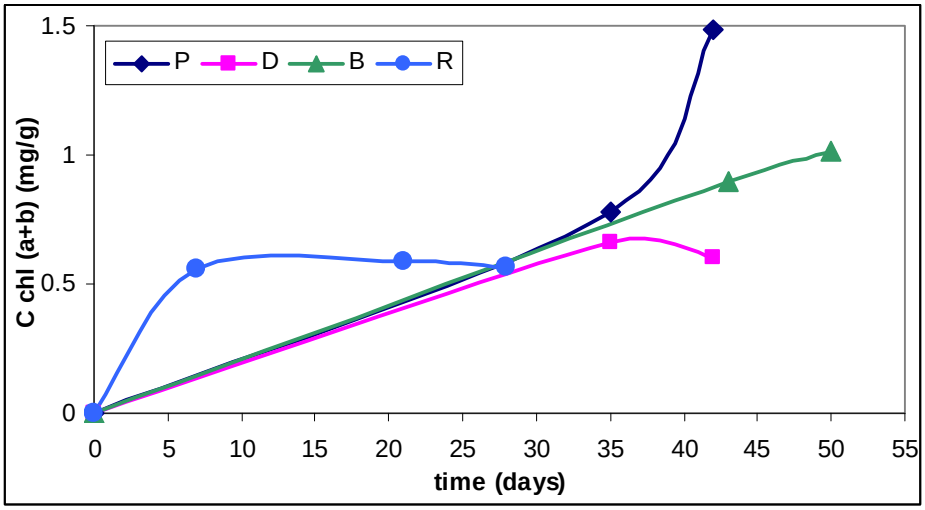

Figure 7. The chlorophyll (a+b) accumulation in species analyzed.

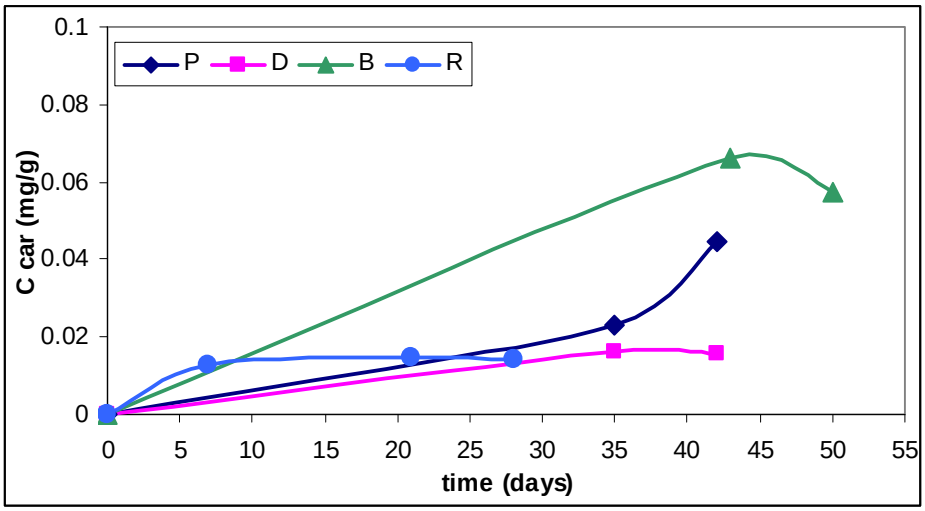

Figure 8. The carotenoid pigments accumulation in species analyzed.

2.3. Chlorophyll and carotenoid pigments content

Determination of chlorophyll and carotenoid pigments was done by the Schöpfer's method (1989).

For determination of chlorophyll and carotenoid pigments was used plant material powder.

In order to differentiate between chlorophyll a, chlorophyll b and carotenoid pigments, the color intensities of the supernatant obtained after the extraction from plant material, was read at the following wavelengths: 480, 647, 652, 663 and 750 nm. The reading at 750 nm is only to ensure that such pigments absorb and do not lead to obtaining inaccurate results. The content of chlorophyll a, chlorophyll b and carotenoid pigments were calculated using the following formulas:

CChl a = [11,78 x E664 – 2,29 x E647] x FD x g sample

CChl b = [20,05 x E647 – 4,77 x E664] x FD x g sample

CChl (a+b) = [20,20 x E645 + 8,0 x E663] x FD x g sample

CCar = [E480 + 0,114 x E663 -0,638 x E645] x FD x g sample

where: FD - dilution factor.

The results will be expressed in mg/g dry weight.

To the dry (up to constant weight) and weighed (50 mg) sample was added 4 ml ammonia solution (80% acetone, 19.5% UPW or redistilled water and 0.5% ammonia solution 25%) and was grinding for 10 minutes at 5000 rpm using an ultra tachometer. The obtained mixture is transfer to a centrifuge tube and kept in a refrigerator until it completes a series of samples which will then be centrifuged for 20 minutes at 4800 rpm.

Samples thus obtained were analyzed using a molecular absorption spectrometer at the above wavelengths.

3. Results and disscussion

3.1. Dynamics of growth and percent of sprouted seeds

Parsley and dill plants were counted and measured at 14, 21 and 28 days after sowing, because it showed slower growth than the radish and beets, which have been counted and measured after 7 and 14 days after seeding.

Sprouting percent values at various periods of time from seeding, for the 4 types of species analyzed are presented in Figure 3. From the graph it is observed that the species analyzed parsley (P), dill (D) and beet (B) sprouts more slowly and at a moderate rate in the experimental conditions, while radish (R) sprout quickly and in a percent higher.

The heights value represented in Figure 4 represents the average of 15 measurements for each species (each 5 measurements/pot). From Figure 4 it can be observed for the period considered that from the species analyzed, the parsley plants had the slowest growth.

3.2. Dynamics of the chlorophyll and carotenoid pigments accumulation under irradiation with the artificial light, in species analized

The chlorophyll a, chlorophyll b and carotenoid pigments accumulation in plants exposed to artificial light irradiation for different periods of time, are presented in Figure 5 to Figure 8.

Relating to chlorophyll (a, b and a+b) content (Figures 5, 6 and 7), it can be observed a rapid accumulation in radish plants but at a moderate content compared with other species analized.

Regarding the accumulation into parsley, dill and beet the figures show a slow increase but with the elevated values finaly, especially for parsley, after 35 days from sowing. This can be explain because the parsley leaves are well developed after 35 days from sowing, thus increasing the irradiated surface of plant.

In the case of radish, although the surfaces of leaves grows, the chlorophyll concentration rests constant, which can be explained by the fact that the tuber begins to grow, too.

Concerning the content of carotenoid pigments can be seen that accumulation in plants is slow. Between species analyzed, as expected, the largest content it has beet.

4. Conclusions

The main conclusion that emerges from this study is that the dynamics of accumulation of chlorophyll and carotenoid pigments in plants exposed to artificial light irradiation depends on the species of plants, but its development stage, which does not mean plant size.

For example, dill has grown in experimental conditions higher than other species, but leaves less developed. Nevertheless, the contents of chlorophyll and carotenoid pigments have been moderate in dill.

5. Acknowledgement

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6. References

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